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OF

MICROSCOPICAL SCIENCE.

EDITED BY

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HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE
AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY
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On a Free-swimming Hydroid, *Pelagohydra mirabilis*, n. gen. et sp.

By

Arthur Dendy, D.Sc., F.L.S.,

Professor of Biology in the Canterbury College, University of New Zealand.

With Plates 1 and 2.

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1. INTRODUCTION.

The remarkable organism which forms the subject of the present memoir was picked up by myself on the sandy beach at Sumner, a small watering-place near Christchurch, on the east coast of the South Island of New Zealand. One evening in October last (1901), while walking on the shore, I saw lying at my feet a small gelatinous object which had evidently just been thrown up by the tide. On placing it in

a glass of sea water I soon saw that it was still alive, and that it exhibited very unusual features, differing widely from any pelagic organism with which I was acquainted. After studying it for some time with the aid of a pocket lens I took it up to my laboratory at Christchurch, and continued my examination of the living animal the same night. Being unwilling to risk the attempt to keep it alive until the next morning, I then killed it by the addition of osmic acid to the sea water, and preserved it in alcohol. It was unfortunate that the lateness of the hour prevented me from making a more exhaustive examination of the living organism, as more light might have been thereby thrown upon its movements and habits; but it seemed best to try and make sure of having it well preserved for minute investigation subsequently, and in this I was fairly successful. The action of the osmic acid was, as might have been expected in the case of so large an organism, very unequal, some of the more superficial parts being much blackened, while the interior was apparently not affected at all, and consequently turned out to be not in so good a condition for minute histological investigation as I could have wished. Had I suspected how complicated and remarkable the structure of the interior really was I might have thought it best to cut the organism in half in order to allow the osmic acid to penetrate, but as it was it did not seem to me desirable in any way to mutilate the unique specimen at that early stage of the investigation.

It was very soon obvious that the organism was an enormous free-swimming hydroid, from the greater part of the surface of which numerous little medusoids were being budded off in groups. Being about to pay a visit to England, however, I postponed the greater part of the investigation until after my arrival, when I resumed the work in the zoological laboratory of the Owens College. It affords me very great pleasure to express my thanks to Professor Hlickson and his staff for the kind hospitality which I received at their hands, and for the valuable assistance rendered to me during the progress of my research.

2. NOTES ON THE LIVING ANIMAL.

The free-swimming hydroid person of *Pelagohydra mirabilis* (fig. 1) is apparently a pelagic organism. The conditions under which it was found, its subsequent behaviour when observed in sea water, and its peculiar organisation, all point to this conclusion. When placed in a glass of sea water in front of a candle (it was too dark to examine it by daylight) it floated near the surface with the narrow proboscis-like portion of the body, bearing the mouth at its extremity, hanging downwards from the much larger balloon-like structure, which I propose to call the "float." The latter, though near the surface, was totally submerged. Subsequently, when placed in a tin can for removal to the laboratory and kept in the dark, the animal sank to the bottom, though still alive. Probably, therefore, it has the power of rising and sinking in the water like other pelagic organisms, and it may be that it always sinks to some depth beneath the surface when it is dark. The general colour of the organism was a very pale bluish tint, and it was of course translucent. The proboscis, however, was pale pink, intensified round the margin of the mouth. The manubria of the medusoids were also pink. During life the hydroid exhibited some slight power of changing its shape, the float being at one time oval (slightly elongated vertically) and at another contracted into a sphere, while the proboscis exhibited considerable power both of contraction, under which condition it became slightly trumpet-shaped at the end, and of flexion. When both elongated, as shown in fig. 1, the float was nearly an inch in greater diameter and the proboscis rather more than half as long as the float.

The long, slender, tentacular processes of the float occasionally exhibited spasmodic movements of flexion, like gigantic flagella, many of them simultaneously, or nearly so; and from this I am led to conclude that the animal has the power of rowing itself through the water by means of these organs.

Whether the medusoids naturally separate from the hydroid I cannot say from direct observation. They exhibited slight twitching movements of contraction, however, while still attached to the parent, and the structure of the larger ones leaves no doubt that they ultimately become free-swimming. Moreover many of them became detached when the organism was killed.

3. THE HYDROID.

(a) External Characters.—The body of the hydroid is, as compared with the ordinary hydroid type—such as we see, for example, in Tubularia,—greatly modified in form and structure, and the modification is such as to bring about the necessary adaptation to the changed conditions of life. The usual stalk is entirely wanting, nor is there the slightest indication of its having ever existed. The aboral portion of the body is enormously swollen out, and quite evenly rounded off at the upper pole, forming the nearly spherical “float.” To the lower pole of the float is attached the cylindrical “proboscis,” bearing the mouth at its extremity. The line of junction between the float and the proboscis is well marked even externally, and corresponds to an even more pronounced internal demarcation between the two.

The float carries numerous long tentacles, which are scattered without any definite arrangement at approximately equal distances from one another all over its surface. These tentacles are cylindrical and bluntly rounded at the extremity, never distinctly knobbed. When fully extended they may be about as long as the float itself. For the most part they are, as usual amongst the Hydrozoa, unbranched, but two or three were observed each with a single branch (figs. 3, 5, *B.T.*), this condition being probably abnormal.

The proboscis is differentiated transversely into two portions (fig. 2). The upper part bears no tentacles, and exhibits an appearance of circular and longitudinal striation,

The lower part, next to the mouth, bears numerous tentacles of greatly varying size; these are arranged, not quite regularly, in transverse rows or whorls, and decrease in size from the uppermost whorl, which contains the largest, towards the mouth, around the margin of which the tentacles are very minute. There is altogether a good deal of irregularity about the size and shape of these tentacles, and here again one of them was found to be branched (fig. 2, *B.T.*), but a better idea of their form and arrangement will be gained from the illustration than from any description which I can give.

Scattered all over the surface of the float, between the bases of the tentacles (figs. 4, etc.), are numerous little branching processes, which we may term "stolons." They branch quite irregularly, their branches remaining short and keeping close to the surface of the hydroid. On these stolons are borne groups of very small medusoids in various stages of development, from minute buds to fully formed bells apparently just ready to separate.

(b) Internal Anatomy.—The most striking feature of the internal anatomy is the presence of two large cavities, completely separated from one another by a thin horizontal septum, as shown in fig. 5. This septum lies at the level of the junction between the proboscis and the float, and is slightly arched upwards. A preliminary examination reveals the fact that the lower and very much smaller chamber is the main gastral cavity, while the upper one is apparently excavated in the enormously developed mesogloea between the ectoderm and endoderm of the roof of the gastral cavity: this second and much larger chamber I propose to call the "cavity of the float." Its real origin will be discussed presently. It is not a simple cavity, but is subdivided by what I propose to term the "supporting membranes." On the inner surface of the wall of the float there is a network of canals, which give it a honeycombed appearance. These canals are lined by endoderm, and are in reality continuations of the gastral cavity, into which they open at their

lower extremities. I shall speak of them as the "endodermal canals."

The Gastral Cavity.—The main gastral cavity, then, occupies only the interior of the proboscis, but is continued upwards into the float in the form of endodermal canals. The lining membrane of the main gastral cavity is thrown into numerous very prominent longitudinal folds, forming ridges which project inwards (figs. 5—9, *L.G.R.*), and whose edges, in the contracted specimen, are very sinuous (fig. 6). At a short distance below the septum the gastral cavity widens out somewhat, and the ridges almost die away. At the junction of the septum with the outer wall of the gastral cavity a prominent annular fold projects into the latter (figs. 7, 8, *A.F.*).

The Endodermal Canals.—Above the fold just mentioned, around the margin of the septum, which is otherwise imperforate, lie the openings of the endodermal canals (figs. 7, 8, *Op. End.*). From the network which these canals form on the inner surface of the wall of the float (figs. 5, 6, *End. C.*) short branches are given off outwards, which run into the stolons; but the canals themselves have apparently no communication with the tentacles (fig. 8).

The Septum.—The septum which separates the main gastral cavity from the cavity of the float is a thin but firm membrane. As already stated, it is somewhat arched upwards. Its two surfaces are both smooth, but to the upper one are attached some of the supporting membranes in the chamber of the float (figs. 5—8). It is, as already stated, imperforate, except for the openings of the endodermal canals, and in this respect differs from either of the two "diaphragms" in the gigantic *Branchiocerianthus imperator*, which in some respects certainly resembles our hydroid.¹

The Cavity of the Float.—The cavity of the float is very spacious, but it is subdivided by numerous very thin,

¹ *Vide* Miyajima, 'Journ. Coll. Sci. Imp. University of Tokio,' vol. xiii, p. 235, etc.

transparent, membranous sheets, which radiate outwards from a more solid mass of tissue formed by their union nearly in the middle of the chamber, and which have their edges attached to the inner surface of the wall of the float and to the upper surface of the septum. These remarkable structures I have called the "supporting membranes." The inner surface of the wall of the chamber exhibits a honey-combed appearance, being marked out into roundly polygonal areas by the projecting endodermal canals. In the centre of each depressed area between the endodermal canals a knob-like projection may frequently be seen; this is caused by the tissue which fills the cavity of the tentacle projecting inwards into the chamber of the float like a plug (figs. 6, 8, *Ten. Pl.*). These structures we may call the "tentacle plugs."

The Tentacles.—All the tentacles are filled with a highly vacuolated tissue, composed of sheets or strands of delicate membrane. In the case of the tentacles of the float this tissue may, as just stated, project as a plug into the float cavity. In the proboscis the mesoglœa in the wall of the gastral cavity is, in the neighbourhood of the tentacle bases, much thickened and highly vacuolated, giving rise to cavities of considerable size, and this vacuolated tissue is continued into the tentacles (figs. 8, 9). The exact nature and origin of the tissue which thus fills the interior of all the tentacles are, however, by no means easy to determine, and the question will be best dealt with under the next heading.

(c) Histology.—Pelagohydra exhibits, for a hydroid, a remarkable amount of histological differentiation. For purposes of description it will be most convenient to subdivide this part of our subject according to the different regions of the body, rather than to attempt to follow out each layer completely before passing on to the next. Indeed, as we shall see later, in some parts of the body the delimitation of the layers is by no means always obvious—at any rate, in the case of the endoderm.

As already indicated, the histological preservation of the

internal tissues is not all that could be desired, and it is greatly to be hoped that an opportunity may arise of working out this subject more in detail with the aid of material specially treated for the purpose. It is also highly desirable that a detailed comparison should be made of the histological structure of *Corymorpha*, *Monocaulus*, and *Branchiocerianthus*, which are evidently related to *Pelagohydra*, and, like it, of exceptional size.

Wall of the Proboscis.—The ectoderm is a thick layer densely charged with small, darkly staining nuclei and thread-cells irregularly scattered throughout its substance (figs. 9, 10, *Ect.*).

In section it exhibits numerous fine radial lines running in at right angles from its outer surface, and perhaps indicating the boundaries of a single layer of large prismatic cells. On its inner aspect, immediately contiguous to the mesoglœa, is a well-developed layer of longitudinal muscle-fibres. In transverse sections (fig. 10) we see that this layer, consisting of an approximately single row of fibres, is thrown into longitudinal folds, the mesoglœa being produced outwards in plate-like ridges between the folds. This arrangement, so well known in the mesenteries of the Actinians, no doubt serves to increase the extent of the muscular tissue.

The ectoderm decreases in thickness from below upwards, and the folding of the muscular layer is especially conspicuous just above the region of the tentacles, and dies away as it approaches the upper limit of the proboscis. Between the bases of the proboscis tentacles the ectoderm is extremely thick, but thins out greatly over the tentacles themselves.

The endodermal lining of the proboscis wall is enormously thick, and throughout the greater part of its extent is thrown into prominent longitudinal folds or ridges in the manner already described (figs 5—9). The structure of these ridges (figs. 9—11) is very peculiar. The mesoglœal supporting lamella which divides the endoderm from the ectoderm is not continued into them, and is indeed sharply marked off by

another layer of muscle-fibres, which we may consider to be endodermal in origin. These fibres are arranged in a circular manner at right angles to those of the ectoderm (fig. 10), and the extent of the muscular layer is increased by horizontal folds, similar to the vertical folds of the ectodermal layer. These horizontal folds are, of course, recognisable only in vertical sections, while the vertical folds of the ectodermal musculature are conspicuous in transverse section (fig. 10).

The free surfaces of the gastral ridges bounding the gastral cavity are covered with an epithelium of a very peculiar type (figs. 9—11). It consists of long, slender, columnar cells arranged at right angles to the surface. They have a finely granular cytoplasm and distinct nuclei, and appear in the sections to be collected into small groups, like bundles of cigars, from the inner ends of which delicate wavy fibres run obliquely towards the central plane of the ridge, and thence inwards side by side till they meet the mesogloal supporting lamella, where they probably give rise to the circular musculature.¹ The grouping of the epithelial cells into bundles is, I think, probably a post-mortem condition due to contraction in alcohol. I imagine that the cells are normally arranged so that each is continued inwards into a separate fibre. We may probably regard the endoderm of the gastral ridges as glandular-muscular in function, for no doubt it secretes the digestive fluid. There are no thread-cells in the gastral ridges, nor, indeed, have I seen them in any part of the endoderm. On approaching the annular endodermal fold which marks the upper limit of the proboscis the gastral ridges gradually die away, and their epithelium gives place to that which lines the gastral face of the septum on the one hand, and the endodermal canals on the other (fig. 8).

The mesogloal supporting lamella of the proboscis wall may be regarded as being bounded on the outside by the

¹ Compare the structure of the endodermal villi with their muscle-fibres in *Myriothela* (Hardy, 'Quart. Journ. Micr. Sci.,' vol. xxxii, p. 505).

ectodermal, and on the inside by the endodermal layer of muscle-fibres respectively. It is continued into the folds of the muscular layers, and also into the annular fold of endoderm. It has the usual clear gelatinous appearance,¹ and though everywhere more or less distinct, attains its maximum development in the neighbourhood of the tentacle bases, where it appears to become immensely thickened, and at the same time broken up by large vacuoles into a network of irregular sheets (figs. 8, 9). It may possibly be invaded in this region by cells migrating from the endoderm, as will be described later in the case of the supporting membranes of the float; but this point I have not been able to determine.

Tentacles of the Proboscis.—The larger tentacles of the proboscis are identical in structure with those of the float, shown in transverse section in fig. 15. The outer wall of the tentacle is formed by a single layer of short columnar cells; it is highly vacuolated, and abundantly charged with thread-cells in all stages of development; on its inner face is a well-developed single layer of longitudinal muscle-fibres. A more or less distinct layer of mesogloea comes next, crossed in places by slender strands (of protoplasm?) extending inwards from the ectoderm, while the axis of the tentacle is occupied by an irregular network of sheets continuous with the vacuolated mesogloea of the proboscis wall. Here and there over the surfaces of these thin and apparently structureless sheets are scattered very well-defined bodies, which may be either small isolated cells with small nuclei, or, as I am inclined to think, themselves large nuclei with conspicuous nucleoli. These bodies are flattened against, or perhaps in the thickness of, the septa which separate the enormous vacuoles from one another. When seen en face they are nearly round, and about 0.0125 mm. in diameter. Their protoplasm stains fairly deeply, especially that of the small enclosed body, and is scarcely at all granular. It is note-

¹ It seems probable that the fibrillated character of the mesogloea described by Allman and Miyajima (loc. cit.) in *Branchiocerianthus* may be due to the ectodermal and endodermal muscle-fibres attached to it.

worthy that two of these large nuclei may be found lying close together, side by side, on the same side of one septum, which seems to indicate that each cavity in the axial tissue is not simply the enlarged vacuole of a single cell. Though abundant in the tentacles themselves, the large nuclei are, so far as my experience goes, not to be found in the vacuolated mesoglœa with which the axial tissue of the tentacle becomes continuous in the proboscis wall.

Owing partly to the specimen being somewhat injured in the neighbourhood of the mouth (possibly by being washed about by the tide on the sand, with mouth downwards), I have been unable to make a satisfactory investigation of the minute structure of the smallest tentacles. It is evident, however, that these conform much more closely to the ordinary Tubularian type than do the large ones. This may be chiefly owing to their smaller diameter, which enables the membranous septa to stretch right across transversely and more or less parallel with one another, so as to divide the interior into approximately a single row of chambers, surrounded by a very thick layer of mesoglœa inside the ectoderm. Thus it would seem that the axis of the smallest tentacles is occupied by a single row of large vacuolated endoderm cells as usual. Whether even in the smallest tentacles these axial cells retain their connection with the endodermal lining of the gastral cavity is extremely doubtful. In the case of the large tentacles there is no trace of any connection remaining between the axial tissue and the endoderm of the gastral cavity,¹ and the origin of this tissue must remain doubtful. It has probably been originally derived from the endoderm, but it has become so modified in structure and so completely disconnected that perhaps only embryological research can decide the question.

Wall of the Float.—The wall of the float forms but a comparatively thin shell, enclosing the central cavity with its remarkable system of supporting membranes. The histological characters of the ectoderm (fig. 12, *Ect.*) are very

¹ Compare Miyajima's remarks on *Branchiocerianthus*, loc. cit.

similar to those of the corresponding layer in the wall of the proboscis. It is, however, less distinctly muscular. In the immediate neighbourhood of the tentacles it retains the characters which it exhibits in the tentacles themselves, being comparatively thin, and having the muscle-fibres arranged radially in continuation with the longitudinal muscular layer of the tentacle. Elsewhere the ectoderm is thick and very densely crowded with thread-cells.

The Endodermal Canals.—The lining epithelium of the endodermal canals, directly continuous with that of the gastral cavity proper, is differentiated into two very distinct portions, differing greatly in histological character. The canals are somewhat flattened against the wall of the float; their own outer walls form part of the thickness of the latter (fig. 12), and are lined by a layer of large epithelial cells with rounded club-shaped ends projecting into the lumen. These cells have very large vacuoles and small round nuclei, and their very darkly staining granular contents are collected together in or near their swollen club-shaped ends (fig. 12, *End O.*). They also contain darkly staining spherical globules of various sizes. The epithelium forming the inner walls of the endodermal canals, on the other hand, consists of a single layer of smaller cells, approximately cubical in shape, with small nuclei and only a small quantity of faintly staining, finely granular cytoplasm (fig. 12, *End. I.*).

The Supporting Membranes of the Float.—The thin transparent sheets of membrane which subdivide the cavity of the float (figs. 5—8, 12, *Sup. Mem.*) appear to have a very remarkable structure and origin. Each sheet consists of a thin structureless layer of mesogloea (fig. 13, *Mes.*), thickening at the angles where the sheets meet one another. Spread out on each surface of this mesoglœal sheet is a still thinner layer of finely granulated, frothy-looking protoplasm, containing rounded nuclei irregularly scattered through it (fig. 14). No cell boundaries can be distinguished in my preparations, but the protoplasm appears to form a vacuolated syncytium. It may occasionally be collected or drawn

together into a thick rounded blob or drop, containing many nuclei (fig. 13), but this condition appears to be of rare occurrence. Probably the nuclei multiply by division, as indicated in fig. 14, at *x*. This peculiar tissue appears to originate, in part at any rate, from the inner walls of the endodermal canals.¹ The mesogloal portion of these walls may be very thick, and occasionally little groups of cells (fig. 12, *End. Bud*) may be seen growing into it from the endodermal lining of the canal. These cells have very finely granular contents and small nuclei. Irregular cavities (fig. 12, *D. F. C.*) are apparently developed between them, and gradually enlarge until the nuclei become widely separated, while the mesogloea is reduced to thin sheets separating adjacent cavities from one another, and the protoplasm of the endoderm cells becomes spread out over these sheets in the form of a granular syncytium.

Sometimes, where a comparatively thin layer of mesogloea lies behind the endoderm of the inner wall of an endodermal canal, threads of finely granular protoplasm may be seen stretching at right angles through the mesogloea from the one surface (covered by the finely granular syncytium) to the other (covered by the endodermal cells of the canal wall).

Thus it appears that the supporting membranes of the float originate in a peculiar manner from the endoderm. It is not certain, however, that they do not receive cells from the external ectoderm also, for thread-cells in various stages of development may sometimes be observed in places where the mesogloea is thick, beneath the external ectoderm and doubtless derived from the latter. This inward migration of the cnidoblasts can hardly be looked upon as normal, but if they are able to migrate inwards it seems equally possible that other ectoderm cells may do the same, and possibly eventually take part in the formation of the supporting membranes.

¹ Professor Ray Lankester has pointed out to me that a somewhat similar method of tissue formation has been observed in the "laminar tissue" of *Amphioxus* (vide Pouchet, "On the Laminar Tissue of *Amphioxus*," 'Quart. Journ. Micr. Sci.,' vol. xx, n. s., p. 421, pl. xxix).

The Septum.—The histological structure of the septum which divides the main gastral cavity from the cavity of the float is practically identical with that of the inner walls of the endodermal canals, with which it is directly continuous. On its lower face it is covered by a layer of lightly staining cells with small nuclei and finely granular contents, and this is separated by a moderately thick layer of mesoglœa from the finely granular syncytium which covers its upper surface. Some of the supporting membranes of the float are attached to its upper surface, and probably originate from the septum in the same way as those already described originate from the inner walls of the endodermal canals.

Tentacles of the Float.—The tentacles of the float are histologically identical with the large tentacles of the proboscis, as will be seen by comparison of fig. 15 with the description already given. The peculiar manner in which the axial tissue seems to project into the cavity of the float in the form of a cushion or plug has already been referred to. In the projecting plug, however, when best developed, the network of tissue is made up chiefly of a finely granular frothy syncytium, with very little mesoglœa and small nuclei. In the tentacle itself the granular material is hardly recognisable, the septa (fig. 15, *S.M.T.*) are very thin, and the nuclei (fig. 15, *Nu.*) much larger and of a different character, like those in the proboscis tentacles. Thus the "plug" seems to be to some extent transitional in character between the true axial tissue of the tentacle and the very much coarser reticulation formed by the supporting membranes in the interior of the float. It is not always recognisable as a distinct structure, however, and even where best developed it passes gradually into the axial tentacular tissue beyond, while its apparent histological differences may be in part due to the want of penetration of the osmic acid with which the specimen was hardened.

The endodermal canals come very close to the bases of the tentacles, and we may be pretty certain that the axes of the latter are endodermal in origin, though, as in the case of the

proboscis tentacles, embryological research may be required before we can say exactly how they arise.

The Stolons.—The stolons are simply branching hollow outgrowths of the wall of the float in the neighbourhood of the endodermal canals, which are prolonged into them to their extremities (figs. 8, 12, *St.*). The ectoderm (fig. 12, *Ect.*) is composed of the usual large clear cells, rectangular in longitudinal section, with small nuclei pressed against their dividing walls. At its base lies a feebly developed layer of longitudinal muscle-fibres. Thread-cells are almost entirely wanting. The mesoglœa is thick, and traversed by slender threads crossing from ectoderm to endoderm. The endoderm (fig. 12, *End.*) is simply a continuation of the endoderm which lines the outer walls of the endodermal canals, and, like the latter, is composed of large cells, often with rounded extremities projecting into the central lumen, with enormous vacuoles and darkly staining contents massed together either in the rounded end or elsewhere. They have small nuclei, and in addition contain darkly staining spherical globules of various sizes.

The Thread-cells.—The thread-cells (figs. 16, 17) are of large size. The actual nematocysts or capsules are approximately ovoid in shape, but truncated at the somewhat narrower outer ends, and measure, when fully developed, about 0.0128 mm. in longer diameter. Each one is more or less enclosed in a delicate cnidoblast (fig. 17, *cnb.*). When fully developed the thread-cells lie in the outer parts of the large ectoderm cells just beneath the surface, and the cnidoblast is prolonged inwards to the base of the cell in the form of a long thread—the cnidopod¹ (figs. 16, 17, *Cnp.*). The cnidopod is remarkably distinct and tough, so much so that when the ectoderm of a tentacle has been abraded, so that the large ectoderm cells have disappeared, the cnidopods may still remain projecting from the surface like hairs, with or without the thread-cells still attached to their extremities.

¹ Compare Allman, 'Challenger Reports,' "Hydroïda," Part 2, p. xv, for the use of this term.

I have seen no thread-cells with the threads everted, and have not been able to make out any details with regard to the thread itself. No barbs were visible in my preparations. Smaller thread-cells, in various stages of development, lie in the deeper parts of the ectoderm.

4. THE MEDUSOID.

(a) Structure.—Although no free-swimming medusæ have as yet been observed, there can be little doubt that they normally separate from the parent hydroid. As already pointed out, they exhibit movements of contraction while still attached, and separate very readily in the process of killing and preserving. Moreover none of the medusæ, which were found attached to the hydroid in large numbers, were sexually mature, and the largest were only about 1 mm. in longer diameter of the bell.

In the largest examples the bell is considerably deeper than wide, and nearly square, though with rounded angles, in cross-section (figs. 22—24). The mouth of the bell is still very narrow (fig. 23), probably expanding considerably later on. It is surrounded by the velum, around which the margin of the bell has grown out into four arms or lobes, arranged in the form of a cross, per-radially, corresponding to the angles of the bell. Each of these arms bears five tentacles arranged in a very peculiar manner—a pair of larger ones, a pair of smaller ones, and a very small odd one; the largest being furthest from the mouth, the odd nearest to the mouth, and the remaining pair intermediate in position, as shown in fig. 23. All the tentacles are short, even in the living animal, and they are only very slightly if at all swollen at their extremities. It is possible that the number of tentacles increases as the medusa grows older, but their peculiar and definite arrangement seems to indicate that the full complement is already present. The tentacles are filled with solid endoderm formed in the usual manner, while the arms or lobes upon which they are borne are characterised

by an enormous thickening of the ectoderm, containing numerous thread-cells.

At the aboral apex of the bell is a depression, where the exumbrellar ectoderm dips in to meet an outward extension of the endodermal lining of the gastral cavity. This marks the spot where the young medusa is attached to the stolon (fig. 22, *Z.*).

The manubrium (figs. 22, 25, *Man.*) is large, but does not project beyond the mouth of the bell. Its surface is smooth, and there are no outgrowths at its extremity.

The subumbrellar cavity is, in the middle, somewhat octagonal in transverse section (fig. 25), being produced into four shallow per-radial angles where the ectoderm is attached to the radial canals, and four deeper interradi al angles where it is attached to the endodermal lamella. Immediately beneath the subumbrellar ectoderm cells is a layer of transverse ("circular") muscular fibres, and the entire epithelium with its musculature is thrown into transverse folds, as shown in figs. 22—24. Towards the mouth of the bell the cross-section of the subumbrellar cavity becomes square, the interradi al angles alone remaining.

The gastral cavity immediately above the manubrium is cruciate in transverse section, the four arms of the cross being produced outwards into the radial canals, and the endoderm being greatly thickened between them to form four ridges. In the manubrium itself the gastral cavity is squarish or irregular in section, with a variable number of longitudinal endodermal ridges.

The four radial canals present no features of special interest, nor does the thin endodermal lamella by which they remain connected. Near the margin of the bell they open into the circular canal (fig. 22, *c. can.*), enlarged per-radially in the tentacle-bearing arms and then produced to form the solid axes of the tentacles.

No gonads are yet recognisable, but the ectoderm of the manubrium exhibits a thickening all round about the middle

of its length, which probably indicates the position in which they will subsequently appear.

There appear to be no sense-organs, and I have not satisfied myself as to the existence of a nerve-ring. In life there is a pink spot on the outside of the base of each tentacle group, and the manubrium also is more or less pink in colour.

(b) Development.—The medusæ are developed as hollow outgrowths or “buds” from the branching stolons already described, and each stolon may bear as many as half a dozen at the same time in various stages of development. As soon as one medusoid approaches maturity another bud (fig. 20 A) appears on the stolon close to its point of attachment, ready to replace the first when it falls off.

The youngest buds observed are represented in figs. 20 A and 18 B; each is a single hollow outgrowth of the stolon, composed of ectoderm and endoderm, but the thick mesogloea of the stolon disappears almost if not quite completely in the bud (fig. 20). The ectoderm and endoderm also change their character, becoming much more compact and solid-looking, and staining much more darkly.

In the next stage (fig. 18 c) the endocodon is formed from the ectoderm at the apex of the bud. There is, in the section represented in the figure, some appearance of invagination, but if not at first solid the endocodon speedily becomes so.

The endocodon grows inwards, and at the same time the endoderm invaginates as if pushed before it (figs. 18, 19), forming a deep cup. The bottom of this cup is then pushed outwards again through the endocodon to form the hollow, finger-like manubrium, which makes its appearance very early (fig. 20).

Meanwhile the cells of the endocodon arrange themselves in a single layer over the outer surface of the manubrium, the inner surface of the future subumbrella, and the inner surface of the future velum (fig. 20). These layers are at first in close contact, but ultimately the subumbrellar cavity makes its appearance between them.

While these changes have been going on the original gastral cavity of the bud becomes further subdivided by the union of its inner and outer walls interradially (fig. 21) to form the solid endodermal lamella, thus defining the four radial canals and the circular canal. The ectoderm becomes greatly thickened outside the circular canal, and the tentacles begin to grow out.

Hitherto ectoderm and endoderm have everywhere remained in close contact (figs. 20, 21), but the transparent gelatinous mesogloea now appears and forces the layers apart (fig. 25). About the same time the subumbrellar cavity is developed and the velum is ruptured in the middle (fig. 20, w.), giving rise to the mouth of the bell (fig. 23).

5. DISCUSSION OF RESULTS, RELATIONSHIPS, ETC.

Pelagohydra mirabilis is a remarkably interesting organism from several points of view. In the first place it forms an excellent example of adaptation to changed conditions of life, showing us how a representative of a group whose members are normally attached, in the hydroid phase, to the ends of fixed stalks may become adapted to a free-swimming pelagic existence. In the second place it exhibits remarkable structural features, especially in the complication of the gastral cavity with its endodermal canals, and the development of the float with its extraordinary supporting membranes. It also has very striking histological peculiarities, showing in this respect a degree of differentiation perhaps unequalled in any other hydroid.¹

As a pelagic member of a typically non-pelagic group of animals we may compare it with *Pelagonemertes* amongst the Nemertines, *Tomopteris* amongst the Annelids, and *Pelagothuria* amongst the Holothurians, and it may

¹ The gigantic *Branchiocerianthus imperator* probably resembles *Pelagohydra* closely in histological features, but requires further investigation (vide Miyajima, 'Journ. Coll. Sci. Imp. University of Tokio,' vol. xiii, p. 235, etc.).

possibly throw some light upon the origin of that remarkable pelagic group of Hydrozoa the Siphonophora, although it will perhaps hardly bear close comparison with any known member of that order.

That it is an aberrant Tubularian hydroid there can, I think, be no doubt, and its nearest relations appear to be the enigmatical *Corymorpha* and its allies.¹ In the genus *Corymorpha* we also find that there is no true stalk, and the curious prolongation of the body by which the animal fixes itself in the sand or mud is, I believe, homologous with what I have termed the float in *Pelagohydra*. In *Corymorpha* also we have a system of endodermal canals forming a network around a spongy central mass, and communicating at one end with the main gastral cavity. Then, again, in *Corymorpha* curious processes are given off from the surface of the body in the neighbourhood of the endodermal canals, which may be homologous with the stolons of *Pelagohydra*, or possibly with the tentacles of the float. Little is known, however, of the minute anatomy and histology of *Corymorpha*, and a careful investigation in comparison with *Pelagohydra* is greatly to be desired. There are, of course, sufficiently striking differences between the two forms, but these are of a more superficial character, and mainly to be accounted for by the difference in mode of life. Instead of a float we find in *Corymorpha* a kind of rooting process, and the tentacles are confined to one end of the elongated body, where they are arranged in a proximal and a distal set, the latter obviously representing the tentacles of the proboscis in *Pelagohydra*. The position of the stolons, between the two sets of tentacles, is totally different; and the medusæ also are quite distinct, for in *Steenstrupia*, the medusa of *Corymorpha*, we find a single odd tentacle, representing one only of the four tentacle groups of the *Corymorpha* medusa. In both cases, however, the medusæ are markedly quadriradiate, and essentially similar in internal organisation; while in *Amalthæa*, which appears to

¹ Allman, 'Tubularian Hydroids,' p. 386, etc.

be closely related to *Corymorpha*, all four tentacles are developed.

It is a very curious fact that two distinct genera of Tubularian hydroids agreeing in such striking anatomical peculiarities should have become adapted to two such different modes of life, the one swimming freely in the open ocean, and the other rooting itself in the sand at the bottom. It would indeed be difficult to find a better example of the powers of adaptation to divers conditions of life. So far as I am aware there is no other hydroid yet known which has become specially adapted to a pelagic mode of life. It is true that floating hydranths—*Acaulis* and *Nemopsis*—are known, but these have probably become detached from stalks, and are not structurally adapted to a free-swimming existence.

6. DIAGNOSIS OF NEW GENUS AND FAMILY.

Genus *Pelagohydra*, n. gen.—Hydroid solitary, free-swimming; the proximal portion of the body modified to form a float, supported internally by a system of radiating membranes of endodermal origin; the distal portion forming a flexible proboscis, with the mouth at its extremity. Gastral cavity continued from the proboscis into the float in the form of endodermal canals, from which arise branching stolons. Tentacles filiform, scattered over the surface of the float and in whorls around the mouth. Medusæ developed on stolons between the tentacles of the float; quadriradiæ, symmetrical, probably with gonads in the wall of the simple manubrium; tentacles in four per-radial groups of five (possibly more in the adult).

The genus may be regarded as belonging to a distinct family, for which I propose the name *Pelagohydridæ*, and for which the generic diagnosis may at present suffice. This family is, however, closely related to the "*Corymorphinæ*" of Delage and Herouard;¹ indeed, some zoologists might

¹ 'Traité de Zoologie concrète:' "Les Cœlenterés," p. 88.

prefer to modify and extend their conception of the Corymorphinæ so as to include Pelagohydra (as the authors referred to include the Hybocodonidæ and Monocaulidæ of Allman) in preference to making a new family for its reception.

7. DESCRIPTION OF PLATES 1 & 2.

Illustrating Professor Dendy's memoir on "Pelagohydra mirabilis."

EXPLANATION OF LETTERING.

A. F. Annular fold of endoderm around the margin of the septum. *B. T.* Branched tentacles. *C. Can.* Circular canal. *Cnb.* Cnidoblast. *Cnp.* Cnidopod. *D. F. C.* Developing float cavities. *Ecn.* Endocodon of medusa bud. *Ect.* Ectoderm. *End.* Endoderm. *End. Bud.* Buds of endoderm growing into the mesogloea from the inner walls of the endodermal canals. *End. C.* Endodermal canal. *End. I.* Endoderm of inner wall of endodermal canal. *End. L.* Endodermal lamella of medusa. *End. O.* Endoderm of outer wall of endodermal canal. *E. U. E.* Exumbrellar epithelium of medusa. *Fl.* Float. *G. C. Man.* Gastral cavity in manubrium. *L. G. R.* Longitudinal gastral ridges of endoderm. *Man.* Manubrium. *Med.* Medusæ in various stages of development. *Mes.* Mesogloea. *M. F. Ect.* Ectodermal muscle-fibres. *M. F. End.* Endodermal muscle-fibres. *Mo.* Mouth. *Nu.* Nucleus. *Op. End.* Openings of endodermal canals into gastral cavity. *Pr.* Proboscis. *R. Can.* Radial canals. *Sep.* Septum between the main gastral cavity and the cavity of the float. *S. M. T.* Internal supporting membranes of the tentacles. *St.* Stolons. *S. U. C.* Subumbrellar cavity. *S. U. E.* Subumbrellar epithelium of the medusa. *S. U. M.* Subumbrellar muscular layer of the medusa. *Sup. Mem.* Supporting membranes of the float. *Syn.* Vacuolated syncytium covering the supporting membranes of the float. *T. C.* Thread-cells. *Ten. Fl.* Tentacles of float. *Ten. Pr.* Tentacles of proboscis. *Th. A.* Thin area of wall of float around tentacle base. *w.* The point where the ectoderm of the young medusa ruptures to form the opening in the velum. *z.* Nucleus in syncytium apparently dividing. *y.* Point of attachment of subumbrellar epithelio-muscular layer to endodermal lamella. *z.* The place where the medusa was attached to the stolon.

Figs. 1—17 inclusive refer to the hydroid stage of *Pelagohydra mirabilis*; Figs. 18—25 inclusive refer to the medusoid stage of the same.

FIG. 1.—The free-swimming hydroid, from a sketch of the living animal. $\times 2$.

FIG. 2.—External view of a piece cut out of the preserved specimen, showing the arrangement of the proboscis tentacles, etc. $\times 7$.

FIG. 3.—Three adjacent tentacles of the float, showing variation in shape, from the preserved specimen.

FIG. 4.—Portion of the surface of the float, much enlarged, showing the stolons with the developing medusæ, lying between the bases of the tentacles.

FIG. 5.—The preserved specimen after removal of a portion of the wall, showing the gastral cavity, septum, float cavity, supporting membranes of float, endodermal canals, etc. $\times 4$.

FIG. 6.—Internal view of the piece represented in Fig. 2, showing septum, longitudinal gastral ridges, endodermal canals, etc. $\times 7$.

FIG. 7.—Portion of the same turned so as to show the under surface of the septum, with the annular fold of endoderm and the openings of the endodermal canals into the main gastral cavity. $\times 7$.

FIG. 8.—Diagrammatic longitudinal section through a portion of the wall, showing the relations of the internal cavities, septum, endodermal canals, supporting membranes, tentacles, stolon, medusa buds, etc.

FIG. 9.—Part of a transverse section of the wall of the proboscis, through the bases of the larger tentacles and the longitudinal gastral ridges of the endoderm. Drawn under Zeiss objective A, oc. 2, camera outlines.

FIG. 10.—Portion of a transverse section similar to and near the last, to show especially the arrangement of the muscle-fibres. Drawn under Zeiss objective D, oc. 2, camera outlines.

FIG. 11.—Portion of a transverse section of one of the longitudinal gastral ridges, showing the endodermal epithelial cells continued into muscle-fibres. Drawn under Zeiss objective F, oc. 2.

FIG. 12.—Part of a transverse section through the wall of the float, showing an endodermal canal continued outwards into a stolon, and giving rise to supporting membranes of the float by means of groups of cells budded off from its lining epithelium. Drawn under Zeiss objective C, oc. 2, camera outlines (slightly diagrammatic).

FIG. 13.—Part of a transverse section of a supporting membrane from the interior of the float, showing the mesogloæal layer covered on each side by a syncytium, here collected on one side into a rounded multinucleate mass of protoplasm. Drawn under Zeiss objective F, oc. 2, camera outlines.

FIG. 14.—Surface view of one of the supporting membranes of the float,

showing syncytium and nuclei. Drawn under Zeiss objective F, oc. 2, camera outlines.

FIG. 15.—Part of a transverse section of a tentacle from the float. Drawn under Zeiss objective D, oc. 3, camera outlines.

FIG. 16.—Part of the ectoderm layer from a section similar to the last. Drawn under Zeiss objective F, oc. 2, camera outlines.

FIG. 17.—Two thread-cells with their cnidoblasts and cnidopods, from one of the tentacles of the float. Drawn under Zeiss objective F, oc. 2.

(In Figs. 18—21 inclusive, showing stages in the development of the medusæ, the histology is, for the sake of clearness, rendered diagrammatically; the endoderm is shaded; the external ectoderm is unshaded, and the ectoderm of the endocodon and its derivatives is unshaded but has the nuclei represented by dots. All are drawn, with the aid of the camera lucida, under Zeiss objective D, oc. 2.)

FIG. 18.—Two young medusa buds seen in longitudinal section,—B before the formation of the endocodon; C with the endocodon and manubrium developing. (Owing to slight obliquity of the sections, the cavity of the stolon is not shown.)

FIG. 19.—Slightly older medusa bud in longitudinal section.

FIG. 20.—Still older medusa bud in longitudinal section, with a very young bud also springing from the same stolon at A.

FIG. 21.—Transverse section of a medusa bud a little older than the last, showing the radial canals, etc.

FIG. 22.—Side view of one of the oldest medusæ found. Drawn from spirit specimen under Zeiss objective A, oc. 1, as a transparent object.

FIG. 23.—Oral view of similar specimen under similar conditions. The mouth of the bell is now visible in the middle of the velum, between the four tentacle-bearing arms.

FIG. 24.—Aboral view of similar specimen under similar conditions, showing the four radial canals, subumbrellar musculature, etc.

FIG. 25.—Transverse section of a medusa of about the same age. Drawn under Zeiss objective A, oc. 3, camera outlines.

NOTE.—The microscopical sections were all stained with borax carmine.

Studies in the Retina.

Parts III, IV, and V, with Summary.¹

By

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With Plates 3—5.

PART III.

The Migration of the Retinal Nuclei.

IN this third part I had hoped to have dealt further with the material absorbed by the rods from the pigmented epithelium; two important points, however, demand immediate attention. In Part I, in referring to the migration of the nuclei, I slightly misquoted Borysiekiewitz's observations, and in Part II I left a serious gap in the description of the outer ends of the developing rods. They were shown in the figures (e. g. Pl. 31, fig. 29) as if truncated, just, indeed, as they appeared in the sections. This gap I am now in a position to fill (see Part IV), while Part V will describe the fate of the absorbed pigment.

Referring to the migration of the nuclei from the middle nuclear to the outer nuclear layer in Part I (p. 44), astonishment was expressed that it had not been noticed before. The only observer who, so far as I am aware, had called

¹ For Parts I and II see this JOURNAL, vol. xliii, 1900, p. 23, and vol. xlv, 1901, p. 443.

attention to the phenomenon is Borysiekiewitz.¹ This writer recorded two evidences of migration ("Ortswechsel") of nuclei in the human retina. Nuclei wandered outwards—(1) from the outer nuclear layer into the basal limbs of the cones, an observation which was not new; and (2) from the middle nuclear layer through the outer reticular layer. I then added that in this latter case "it was the characters of the migrated nuclei, exactly like those of the layer they had left, and not at all like those of the layer into which they had moved, which convinced him that migration must have taken place." The similarity of these nuclei had been so often noticed by myself as a convincing proof that the nuclei embedded in the outer reticular layer were passing through it, to become transformed into rod nuclei, that after reading Borysiekiewitz's two treatises, and finding that he had also noticed the migration, I inadvertently attributed to him an observation which, however, he does not make. He only indirectly indicates it in his quotation from Dogiel, who recognised a layer of "subepithelial nerve-cells" in the outer nuclear layer, i. e. a layer of "cells" exactly similar to those on the other side of the outer reticular layer, called by Dogiel² "the bipolar cells of the ganglion retinae." Borysiekiewitz remarks that such cells are probably merely his migrated nuclei,³ but rightly adds that they do not form a "layer."

Borysiekiewitz was himself convinced of the migration of the nuclei by finding a tract (l. c., p. 37) in one of his preparations in which the middle nuclear layer changed from two rows into one row and then back again; but where it was in a single row, the missing nuclei were visible either in, or on the outer side of, the reticular layer (for a parallel case see fig. 19, with description). This valuable observation shows

¹ 'Weitere Untersuchungen über den feineren Bau der Netzhaut,' Wien, 1894.

² 'Archiv f. mikro. Anat.,' 38, p. 317.

³ Borysiekiewitz uses the word "Korn," which does not exactly mean nucleus, but in this connection it is practically the nuclei alone about which anything can be definitely stated. The point will be dealt with in my next paper.

that the migration of the nuclei from the middle nuclear layer into the outer, which I described for the Amphibia, occurs also in the human retina; and indeed, I may add, it occurs in all the vertebrate eyes I have yet examined.

Borysiekiewitz's own theory of the essential structure of the retina, in the light of which this migration finds no explanation, is very different from mine. According to him, these nuclei are inside the "Müller's fibres," in the more fluid axial portions of which they can move. The outermost ends of these radial fibres are, according to his view, the rods and cones. So that the migration of the nuclei beyond the outer reticular layer is a kindred phenomenon with their movement beyond the *membrana limitans externa* into the basal limbs of the "cones," both being mere shiftings outward along the axes of the "Müller's fibres." The comparative study of the "Müller's fibres," which will be found in Part V, makes the acceptance of this description impossible. I may add that the only difference which Borysiekiewitz can see between the rods and cones of the human retina, is that the latter are those tips of the Müller's fibres into which nuclei have migrated beyond the *membrana limitans externa*. There is no observable difference in the lengths of their outer limbs.

Confining ourselves to the migration of the nuclei, we may review the position of the argument as far as it was advanced in Parts I and II. In the Amphibia migration from the middle to the outer nuclear layer can not only be seen—(1) in the actual passage of nuclei through the outer reticular layer (Part I, Pl. 3, fig. 5, *e, f*, and Part II, Pl. 31, figs. 23, 24, 25), and (2) in the exact similarity of those in the outer nuclear layer which are not yet rod nuclei, but still close up against the outer reticular layer with certain nuclei in the outermost edge of the middle nuclear layer (see Part II, Pl. 30, fig. 16, *b*), but is a necessary assumption in order to account for the number of new rods required by the growing retina. A short migration within the outer nuclear layer can be seen in the fact that the "cone" nuclei, as the cones assume the definitive rod-form, move outwards from near the outer

reticular layer towards the *membrana limitans externa*. The only possible escapes from the assumption that when the original supply in the outer nuclear layer has been exhausted, fresh supplies migrate outwards from the middle nuclear layer are two: (1) if it could be shown that the layer of rods and cones with their nuclei grows only at the edges; and (2) if it could be shown that the nuclei for the new rods are produced by the division of those already composing the outer nuclear layer.

With regard to the former of these alternatives, it is certainly true that the retina as a whole does grow mainly at the edges. I am not, indeed, now inclined to lay very much stress upon the argument used in Part I, that if growth took place only at the sides, the eye would not keep its shape, for growth at the sides alone would, I thought, merely carry up those sides, and the eyes would be funnel-shaped rather than round cup-shaped. This argument would perhaps hold if no other factors were present which could help to keep the retina hemispherical. There is, however, another traceable factor, the full force of which I did not then see. I refer to the vitreous humour which, as a collection of semi-fluid matter in the hollow of the eye, would, if the supply is kept up at any pressure, compel its flexible walls to adopt the normal shape. But though this is a possible factor in keeping the growing retina round, the argument which refers the persistence of its shape to its own growth-processes can hardly be put altogether on one side, for it is a fact that cones, i. e. new rods, can be seen forming over the whole *Amphibian* retina at all stages of its growth, and even in the eyes of adults.

Secondly, the suggestion that the nuclei for new rods might be supplied by the division of those already present can be met by a decided negative. In embryonic eyes (*Mammalia*), or in amphibian eyes before they are functional (see fig. 5), i. e. before any rods are formed, and only the merest traces of vesicular protrusions are to be seen, divisions of nuclei occur over the whole retina in the outer-

most layers. But as soon as and wherever vesicles are produced and rods begin to be developed out of them, a process which always takes place first in the centre of the retina and spreads outward from the centre, no divisions normally take place. In order to ascertain this point, I have examined the retinas of tadpoles (toads and frogs) killed at almost all hours of day and night.¹ Nuclear divisions were very numerous in the tadpoles killed in the night, and sometimes in those killed in the daytime. A study of them makes it quite safe to affirm that nuclear division is normally confined to the edges of the retina, that is, to those parts where there are no traces, or only the faintest traces, of vesicular protrusions, although one may just occasionally be seen dividing a short way within the zone where the vesicular protrusions are beginning.² This result is obtained from so many amphibian retinas that I have no hesitation in affirming that after the rods have begun to develop, nuclear divisions are never found normally in the layer of rod nuclei. This is, of course, what we should have theoretically expected, that cells specialised for some active function are incapable of mitotic division.

We are, then, debarred from finding the source of the nuclei for new rods in the nuclear layer itself. Hence the new nuclei required must come into the outer nuclear layer from without, i. e. from the middle nuclear layer, by migration through the outer reticular layer; and these migrating nuclei, whether the retina grows mainly at the edges or not, must be many thousands, considering the great numbers of "cones" found in the central regions in all stages of its growth.

This, then, brings us face to face with the question, Whence does the middle nuclear layer obtain the large supply necessary to furnish the outer nuclear layer with so many? No one will suggest that the supply could be kept up from the "ganglionic cell" layer, which in the central regions is

¹ Viz. at almost every hour of the night, from 4 p.m. to 6 a.m.

² They are occasionally found in young fish retinas, even within the already functioning central region!

seldom more than one deep. It is true that nuclei from this layer do pass through the thick inner reticular layer to the middle nuclear layer. Most sections will show, as has been often noted before and variously interpreted, one or two actually within the inner reticular layer. Further, all sections show a number of nuclei in the innermost rows of the middle nuclear layer so like the "ganglionic cells" that they have been recently freely claimed as being "ganglionic," i. e. as of much the same functional activity as the nuclei of the innermost layer, which has always been the "ganglionic cell" layer of authors. Although this resemblance need not necessarily have anything to do with the question of migration, there cannot, to my mind, be any doubt but that the "ganglionic cell" layer is drawn upon by the middle nuclear layer, and may, indeed, for considerable tracts, be quite exhausted (compare fig. 22, *g.l.* in *a*, *b*, and *c*). But such a supply, at its best, would be insufficient to counterbalance the drain on the middle nuclear layer. Further, as in the case of the layer of rod nuclei, no mitotic divisions are found in the "ganglionic cell" layer after the eye has once become functional.¹

We have therefore to seek elsewhere for the supply of nuclei required by the middle layer to enable it to send so many outwards through the outer reticular layer to become the nuclei of the new rods. One would think the most probable source for these nuclei would be the division of those already composing the layer, but here again we are baffled, for divisions do not occur, at least near or in the places where they are wanted. Indeed, the primary object I had in view in examining retinas killed at all hours of the night was to ascertain whether it was not possible that, as no divisions were ever seen in this layer in retinas killed during

¹ I have seen a few traces of fragmentation which deserve attention, but hardly wide-spread enough to meet the present difficulty. See also Borysiewicz's figures ('*Untersuchungen über den feineren Bau der Netzhaut*,' p. 19, 1887), which represent "twin ganglion cells." They certainly suggest divisions of these cells, but are capable of a different interpretation.

the day, they might take place during the night, when the eye is at rest. This, however, as above stated, proves not to be the case; no divisions occur in the middle nuclear layer, except near the edges of the retina, where it is not possible to speak of the middle layer because the two reticular layers which separate the retinal nuclei into zones only begin where the rods and cones are themselves commencing to form.

We have, then, no other source, except this undifferentiated rim, for the enormous number of nuclei required by the middle nuclear layer in order to keep up the supply of rod nuclei required by the growing retina. A few, one here and there, as we have seen, may be obtained from the innermost, or the "ganglionic cell" layer, but none from divisions of those already present. The real supply must come, as stated, from the rim of the retina; and however startling the idea may at first appear, we have to assume a stream of nuclei from the undifferentiated edges of the retina towards the base of the cup. Further, as long as growth lasts, this streaming must be considerable, for in addition to the supply of nuclei for the formation of new rods, the thickness of the middle layer is kept up,¹ even though the layer itself has to expand greatly as the eye grows larger. Indeed, it has not only to extend as the eye grows, but, as compared with the bulk of the layer in very young eyes, it may also greatly thicken. Sections of small retinas (of tadpoles) about 0.5 mm. in diameter may show the middle layer in the central region only three nuclei deep, while eyes over 1 mm. in diameter may show it six nuclei deep; in the adult frog a layer four deep is very common. But I do not think that much importance can be laid upon these variations in thickness, as they are probably accidents of nourishment and growth. It is quite possible that at times the supply of fresh nuclei may be greater than the immediate demand, in which case the layer would temporarily thicken; or in times of bad nourishment

¹ Apparently in all eyes, except in the "fovea centralis" of human and ape retinas.

the supply might be less than the demand, and the layer in consequence thin away. The really important fact is that we find ourselves compelled to assume a migration of nuclei on a very large scale. Not only can it be shown that nuclei travel outwards through the outer reticular layer in great numbers to become rod nuclei, but that all the nuclei destined for this function have, at least after the original supply has been used up, to travel down from the edge of the retina along the middle nuclear layer to their ultimate destinations. In addition to these movements it can be shown that nuclei of the so-called "ganglionic cell" layer occasionally travel outwards through the thick inner reticular layer until in old eyes (*g.l.*, fig. 21) they may be almost entirely used up.

Our investigations into the growth-processes of the retinas of some score of frog- and toad-tadpoles having thus eliminated all other possible sources for the nuclei of new rods required by the central regions of the retina except this immigration from the rim, it remains for us to see what direct or indirect evidence there is for such an unexpected phenomenon, not as an occasional, but as a normal growth-process. It is hardly likely that such a movement could take place without showing visible traces,—without leaving its mark on the tectonics of the retina itself. We shall now see that this surmise is fully justified.

The divisions take place in early growth-stages along the whole of the rim into the iris, but are most numerous in the angle between the iris and the cup of the retina. To this angle, as the iris becomes differentiated, they are usually confined. They also take place chiefly, though not exclusively, in the outermost row of nuclei, in what I have elsewhere called the palisade layer. In the part where the divisions are active it is common to find the large, radially arranged, more or less spindle-shaped nuclei attached either to the internal or to the external limiting membrane by a frequently thick staining cytoplasmic strand. The nuclei are usually so numerous as to obscure the sections, so that one cannot state that these strands, each with its suspended nucleus, run distinct and

isolated from membrane to membrane. All that is really important at the present moment is to note that the nuclei in the region of active division are attached to one or other of the "limiting membranes" by definite strands which are only found in this undifferentiated rim.

Now these strands can often be seen showing the following interesting arrangement:—On the axial side of this area of nuclear division, and just where the differentiation of the retina into zones is commencing, the nuclei, still for the most part having retained their spindle shapes, are seen to be arranged in slight curves (figs. 1 and 2); the two ends of the curves are attached by these strands to the limiting membranes, and their middle parts bulge outwards towards the axis of the eye. This curving might easily be passed over, and when seen it might be considered as a purely accidental phenomenon. It is far more probable, however, that it is normal, and due to the process we are discussing, viz. the tendency of the nuclei to travel from the rim towards the functional axial region of the retina. It is clear that the curving could be so explained.

Again, comparisons of the different thicknesses of the middle nuclear layer at different parts of the retina and at different stages in its growth tell the same story of movement. We always find that the layer is thickest near the rim where the nuclei produced by division are crowding into it, and thinnest in and near the centre where the nuclei are presumably in most demand. Further, the variations in thickness of this layer in the central region at different stages of growth clearly show fluctuations in the numbers and changes in the positions of its component nuclei.

If the nuclei from the undifferentiated rim have, then, this tendency to stream inwards towards the axis, it is clearly those occupying the middle ranks in the retina which would be the freest to move, and which therefore would travel fastest. Those of the innermost ranks will be more firmly attached to the internal limiting membrane, and may, perhaps, be further entangled by the developing nerve-layer,

while externally the nuclei are for the most part functioning as rod nuclei. It would thus be only a band of nuclei down the middle which would be freest to travel towards the axis. This fact gives us a clue to the origin of the zonal arrangement of the retina into alternating nuclear and reticular layers.

It is fairly clear that if a band of nuclei travelled along between two stationary layers such as the innermost and outermost layers in an amphibian retina, and if, when they started, they had cytoplasmic attachments to the limiting membranes, they would almost certainly leave traces of those attachments trailed along on each side of the stream, and the accumulations of the trailings would separate them from the stationary fringing layers. We get, indeed, in this somewhat startling and unexpected manner a perfectly intelligible reason for the existence of the two reticular layers. It is unexpected because, considering that all these cytoplasmic strands are living protoplasm, it would appear more natural if they had readjusted themselves in the retina, letting the nuclei pass on. The evidence, however, shows clearly that this is not the case, and that they are to a large extent trailed along and assist in the formation, at least, of the inner reticular layer. I say "assist" because they apparently only form its cytoplasmic basis; other elements, as we shall see later on, contribute to the final result.

In studying thin sections of retinas of tadpoles I had often been struck by the fact that from the extreme end of the inner reticular layer irregular threads went off and curved inwards towards the *membrana limitans interna*. This is more striking in some cases than in others. Figs. 1 and 2 are sufficient to show what is meant, but in some cases I have seen it so marked that it looked as if the inner reticular layer took its origin, at each end of the section, from the internal limiting membrane, sometimes almost shutting off the layer of "ganglionic cells" from those of the undifferentiated rim. This appearance greatly puzzled me until the discovery of the migration of the nuclei made it clear that these threads which joined the inner reticular

layer to the internal membrane were the remains of the attachments of the originally spindle-shaped nuclei which had moved away down the middle nuclear layer.

More conclusive still is the fact that every now and then a section is found in which the nuclei of the middle layer, especially near the rim, are actually caught trailing irregular tangles of cytoplasmic threads in the manner shown in figs. 1 and 2. This can be seen with some frequency, though by no means always, because it is probably a matter of accident whether the particular retina happened, at the moment it was fixed, to be in the exact phase of its life activities which required such movements. For it is hardly likely that the inward streamings of nuclei are continuous; periods of rest would probably intervene. However seldom they occur there is no mistaking their significance.

Still keeping the movement of the nuclei in view, it is worth while paying further attention to the inner reticular layer. We find that the early stages in its appearance show differences which, though at first disconcerting, are yet on the whole entirely confirmatory. The earliest stages which I have so far seen are shown in figs. 5, 6, and 7, which I interpret as follows:—The nuclei, which had been fairly evenly distributed through the retina, and not tightly squeezed together (see fig. 7), gradually separate along the line which will be later occupied by the inner reticular layer, the larger half migrating outwards. A row, two or three deep, remains against the internal limiting membrane, although one or two even of these, in the axis of the eye, may escape outwards, leaving a gap in the innermost layer (figs. 5 and 6). The great mass of the nuclei gradually move, as stated, outwards, but the very outermost can at the most move but a few micromillimetres, being arrested at once by the pigment epithelium. The rest, therefore, leaving a few stragglers, crowd up close behind, with the result that the irregular but conspicuous rent, just described, occurs in the previously uniform nuclear ranks. This rent in its early stages seems to be mainly occupied by rounded vesicles, at least in the retina from which fig. 7 was

drawn, but later becomes filled with a rather loose tangle of staining matter, composed mainly of the cytoplasmic framework in which the nuclei were suspended. These phases not only reveal the outward movement of the nuclei, but also show that it is not due to pressure such as might be exerted from a region of active division. The nuclei in these sections can only have moved outwards under the action of some attraction. This fact, that the force bringing about these migrations is attractive, is important, though we cannot stop at the present moment to develop it further.

A slightly later phase in the formation of the inner reticular layer can be seen in figs. 3 and 4. The irregularities seen in the layer in its first appearance, as a reticulum filling up a split among the ranks of the nuclei, as just described, have become more definable as tongues running out among the outwardly pressing nuclei. In one case (fig. 4) several tongues appear of nearly equal size, although the one which appeared to be nearly in the axis of the eye was the largest. In another case (fig. 3) this axial tongue was very much larger than any of the others. As will be seen from the direction of the arrows in this latter figure, I explain these phenomena as due to the migration of nuclei from the sides. The attraction which first drew the nuclei from their original positions in the embryonic retina to press outwards has extended and drawn nuclei from the peripheral portions of the retina which have not yet begun to function. This combined centripetal and outward movement of the nuclei would naturally give the rudiments of the inner reticular layer the shapes which they assume in these sections. That this movement is taking place in the direction of the arrows may be gathered from the closeness with which the nuclei in these sections are packed in the central and more actively functioning region as compared with their straggling and loose arrangement elsewhere.

We have to add to this evidence, each item of which seems fairly conclusive, the fact that the inner reticular layer grows thicker as it slowly reaches the adult condition, and not

only thicker but very much more extensive without the appearance of any special formative cells which would account for it.¹ If, however, the nuclei of the middle layer, each with more or less cytoplasm trailing behind it, do actually move along from the rim of the retina towards the axis, we can account not only for the gradual thickening of the inner reticular layer as the eye grows, but also for its curious stratification, which is sometimes very striking. The layer reaches its definitive thickness when the eye has ceased to grow and no more nuclei are produced at the rim.

So far, however, we have only considered the inner reticular layer, but there are two such layers, as there should be if the mechanics of their formation here sketched be correct. If correct, it supplies us also with an explanation of the fact that the two reticular layers are always co-extensive with the region of rod-formation, only appearing where the vesicles are being protruded. A slight difficulty, however, now arises. If these layers are produced by the nuclei travelling down the middle layer from the rim towards the centre, why is not the reticular layer on the outer side of the stream as thick as that on the inner? An answer may be suggested which is probably correct, although it would be difficult to bring any evidence for or against it. Great numbers of the nuclei travelling on the outer side are arrested as they go and pass into the layer of rod nuclei. These might be expected to take all the cytoplasm they could with them as the formative substance of the vesicular protrusions which they are destined to send out from the retina for the formation of their rods. Hence it is probable that the greater part of the cytoplasmic reticulum which would otherwise be accumulated here as a counterpart of the inner reticular layer is carried outwards and used up

¹ I have never seen any indication of the rows of small, faintly outlined, formative cells such as Borysiekewitz (l. c.) describes for the inner reticular layer in human retinas; whenever nuclei do occur in the layer, in all the eyes I have examined, they are always quite distinct, and to be regarded as migrating outwards from the "ganglionic cell" layer.

in the production of rods. This seems to be a possible explanation of the difficulty. Some traces of an outer reticular layer, however; there always are, and doubtless here again the retinal cytoplasm forms its basis, and only its basis. In a future paper I shall show that neither of the reticular layers is a homogeneous structure; the outer layer, indeed, presents several difficult problems. In fig. 3, *o.r.*, we already see signs of accumulations of deeply staining matter along the line of the future outer reticular layer. These accumulations, which we shall meet with again in Part V, are apparently in some way due to the functioning of the nuclei, for it is obvious they cannot, from their position, be due to any merely mechanical streaming movements.

In the very existence of these two reticular layers, as well as in their stratified texture, in their attachments round the rim by threads to the *membrana limitans interna*, and in the shapes they assume during early growth, we find strong evidence of the migration of the nuclei, which is the subject we have specially in hand.¹ We may sum up the arguments briefly: (1) the nuclei of the adult rods protrude a little beyond the *membrana limitans externa*; (2) the nuclei of the cones, which (in *Amphibia*) are early stages in the formation of new rods, move gradually outwards from near the outer reticular layer towards the *membrana limitans externa* as their rods develop; (3) no nuclear division takes place in this layer where rods and cones are developing; the nuclei for the further production of rods come through the outer reticular layer from the middle nuclear layer; (4) no nuclear division takes place in this middle layer anywhere near the axial portion of the retina, and the supply must be kept up by migration from the sides. A very few may come through the inner reticular layer from the layer of "ganglionic cells," but the bulk of

¹ We shall refer to some of the very discordant views which have been put out as to the origin and constitution of these layers when we come to deal with them in detail. In the meantime a useful summary may be found in the *Literatur-Verzeichniss* to Borysiewitz's first paper, 'Untersuchungen über den feinceren Bau der Netzhaut,' 1887, notes 19—27.

those required travel along the middle nuclear layer from the undifferentiated rim of the retina where nuclear division is active during growth. Thus a stream of nuclei travels inwards from this undifferentiated rim towards the axis along the middle nuclear layer; (5) this stream of nuclei lays the foundation for the two reticular layers of the retina; the cytoplasmic trailings of the nuclei being, as it were, swept to the sides of the stream, accumulate, but while the inner accumulation persists the outer is mostly used up, probably in the formation of the rod-vesicles.

Before leaving the subject for the present, I should like to call attention to the conviction which I expressed in Part II, p. 452, that the retina is a syncytium, in the reticulum of which nuclei are suspended, and that it is almost impossible to speak of "cells" in connection with its component elements. The streaming of the nuclei and the trailing behind them of cytoplasmic tangles, which trailings accumulate as the eye grows, may, I think, be regarded as complete justification for this conviction. I had not forgotten and do not forget the large "ganglionic cells," which appear to supply an easy refutation. On the contrary, it was a prolonged study of these same "cells" which first led me to this conclusion, as I shall relate in detail in a future paper.

Lastly, I should like to venture the suggestion that the principle here established for the retina may be of wide application, although I cannot hear of any other exemplification of it as yet known. The principle is this: an organ has to continue to grow after it has begun to function. Assuming that nuclei or cells are incapable of mitotic division when once specialised for some highly complex function, we should be compelled to postulate an undifferentiated region which would persist as long as growth lasts. From this region, which would be the centre of active nuclear or cell division, the new elements required by the functioning and growing area would have to migrate, through longer or shorter distances according to the exigencies of the

particular case. Further, as in the case of the retina, these migrations may have considerable influence on the tectonics of the organs in which they can be established.

PART IV.

On the Vesicular Swellings at the Tips of the "Cones" and some Earlier Form-phases in Rod- production in the Amphibia.

As was shown in Part I,¹ the tips of the young cones swelled into vesicles on reaching the pigment layer. Vesicles or parts of vesicles were figured (Pl. 3, figs. 2, 3, and 10), and these justified the construction of the series of form-changes shown in the diagram (fig. 4), but they were only certainly seen in eyes fixed with boiling corrosive sublimate. Other figures on the same plate (e. g. fig. 12, and on Pl. 31, Part II,² fig. 29) showed no traces of any such vesicular tips, and in some cases it was difficult to understand why, if they had existed, they should vanish so completely from the sections. This point has now been settled, not, I regret to say, by the discovery of a new and more perfect fixative, but by a kind of good fortune. I brought down a few tadpoles from Table Mountain, Cape Town, killed and fixed them in Perenyi's fluid at midnight, i. e. when the pigment would be retracted. The object was to see whether, owing to the brilliant sunlight of South Africa and the intense heat, the pigmentation in the retina showed any modifications on that seen in our indigenous tadpoles, and if so whether any correlated changes in the retina could be discovered. For the same reason I made special efforts to obtain baboon's eyes (see Part V).

One interesting difference was at once apparent. The pigment in the South African tadpoles is far greater in

¹ This Journal, vol. xliii, 1900, p. 23.

² Ibid., vol. xlv, 1901, p. 443.

quantity and of a very much darker brown. The colour is in striking contrast with the reddish brown which is most common over here. But this was not all; probably in correlation with this increase in mass and quality of the pigment the rods were also different (see figs. 8, 9, and 10), in that the longitudinal striation is so marked that it can be seen at once with a low power. Cross-sections of rods which seem to be somewhat thin, $4\ \mu$ across, often tapering to $3\ \mu$, show a thick, straggling, branching, and knotted strand running down the axis of each rod as the representative of the axial reticulum, and connected irregularly with the dark striæ running down the wall. Here and there the greater part of the axis of the rod is taken up by a mass of dull grey homogeneous matter, in which case the axial reticulum is apparently represented by clump \bar{s} at the sides, but it usually comes into view again on focussing up or down. These grey masses in the rods are the remains of material absorbed from the pigment granules (see fig. 8, with description).

Apparently correlated with these strongly developed staining striæ is the fact that the rods, though very thin, are comparatively speaking tough; for, quite unlike those in our own species of *Amphibia*, which break up so easily and usually part at the junction of the inner and outer limbs, in these eyes, where the retina and choroid have parted, they are drawn intact out of the dense pigment.

Turning to the cones, we fortunately find that their vesicular tips share in this greater toughness. Very many of these latter, it is true, have broken down and have been reduced to a granular mash which is very conspicuous, but places such as those figured (figs. 9 and 10) might be multiplied to any extent. The vesicles are shrunken, and it is largely owing to the folds in their walls that they are visible. Some seem to have clear traces of rows of dots running down them which remind one of the rows of dots on the longitudinal striæ of the rods. In optical section the wall of the shrunken vesicle could often be traced quite plainly into that of the cone (fig. 10).

We conclude, then, that in all young amphibian eyes, in which the rod layer seems to consist mainly of cones ending at some distance from the pigment, the apparently vacant space between the truncated cone-tips and the pigment is, in life, filled up by a compact mass of swollen vesicles. These vesicles are, however, so exquisitely delicate that the process of fixation and hardening destroys them almost completely. But I should add that now that I have seen the vesicles in the Table Mountain specimens, I have been able to discover, in sections of our native forms, several cone-tips running out into faint diverging threads.

Another peculiarity in the retinas of these Table Mountain tadpoles deserves mention. In Part I, p. 34, I remarked in a note that the only long-necked elements which I could find in frogs' retinas at all resembling the long-necked "cones" figured by van Genderen Stort ('Quain's Anatomy,' 10th ed., vol. iii, part 3, p. 48) were those which appeared in each case as one of the so-called twin or double cones (see Pl. 3, fig. 5). Besides these, the only elements with long inner limbs were Schwalbe's rods, in which the refractive globule had, as a rule, already disappeared and the outer limbs had already become cylindrical (Part I, Pl. 3, fig. 4, *r*). But in the retinas of these Table Mountain tadpoles, cones with striking refractive globules like those figured by van Genderen Stort are very plentiful, close down against the pigment layer. The greater toughness of the walls may account for the persistence of the shape in a phase where it is quite lost in our native forms (see the phases Part I, fig. 4, *c₄* and *r*). The fact that the refractive globule is not so quickly absorbed may be referred to the great quantities of pigment to be dealt with (for the origin of this globule see Part II, p. 463).

The transformation of these long-necked cones into rods is, in some cases, very easy to follow. The conical portion thickens and shortens, while the swollen vesicle at the tip becomes cylindrical and the refractive globule disappears. In fig. 9 elements like those on the right and left hand are very common; that on the right shows a division in the

material filling the rod; the short innermost darker portion clearly corresponds with the old staining tip of the cone (cf. the sections indicated by asterisks). The small numerals 1-7 show a continuous developmental series illustrating the transformation of long-necked cones into Schwalbe's rods.

It was stated in Part I that the growth-forms (c_1, c_2, c_3) of the new rods shown in Pl. 3, fig. 4, of that paper were due to the fact that each new element on being protruded had to force its way between tightly packed cylindrical rods; obviously the new vesicle could only swell into a sac after getting through, that is, against the pigment. If this explanation be correct we should expect to find other form-phases where the rods were not so long. A much more direct transformation of cones into rods was, indeed, figured in the series of elements supplied by the axolotl, Part 1, Pl. 3, fig. 8.¹ In this animal the rods are very thick, and, compared with their thickness, very short. Now it is interesting to note that we have at the sides of tadpole retinas, where the rods get progressively shorter, a very similar process of direct transformation of cones into rods to that which we found in the axolotl. The distal portion of the protruded cone seems to be neatly rounded off (fig. 11), as if there had never been any swollen vesicle at its tip. It is further quite distinctly striated longitudinally.² Here, then, we have the cones changing directly into rods by the absorption of the refractive globule and the lengthening of the outer limb at the expense of the inner. If we compare this process closely with that occurring among the long rods in the central regions of the retina (figs. 9 and 10); we find that it differs in two points: (1) there is no long thin neck, and consequently (2) there would appear to be no conspicuously swollen vesicle at the tip which would have ulti-

¹ To make that figure true to life the tips of the cones in *a* and *b* should have been drawn with delicate vesicles, but all traces of such vesicles had been destroyed in the actual sections.

² Compare the dots seen on the distal vesicles shown in figs. 9 and 10, also the remarks on the striation of the cones in Part II, p. 455.

mately to be brought into the typical cylindrical form. It is easy to see that both these differences are due solely to the fact that where the rods are long cylinders the protrusion has to force its way between them, and only swells out into a conspicuous vesicle after getting through.

It will be seen from the study of these details how important it is to keep the compactness of the layer of rods very clearly before the mind. The rod layer, in fact, arises as the result of the thrusting out of great numbers of vesicles from the retina, the vesicles only gradually assuming the long, cylindrical rod shape. The varying forms which the early stages of new rods assume when first protruded, and until they are finally developed, depend not only upon the forms, but also upon the lengths of those among which they have to force their way. We have now seen two of these different series of form-changes, and it will be best in this connection to record the observations made on still earlier stages of growth, when the new vesicles are protruded, not among rods, but among other vesicles which have not had time to become rods. We shall see that whereas, when the rods are formed, and their shapes fixed, new vesicles have to adapt themselves entirely to them; while the rods are still unformed and vesicular the protrusion of new vesicles is able to modify their shapes. In the changes described in Part I we saw that the protrusion of fresh cones altered the shapes only of other cones, helping to change long cones into Schwalbe's rods, but that they had no apparent effect upon finished rods.

The first appearance of rod-vesicles begins very early, as soon as ever the eye begins to function. They can be seen in various sizes in figs. 3 to 7, as round clear spaces against the pigment. At first they are scattered and confused, because all the nuclei do not secrete vesicles simultaneously (see figs. 16, 17, and 18). A little later a stage is reached when they are arranged side by side as large sacs mutually compressing one another (figs. 12 and 15). It is at this stage that our sections usually fail us. So long as the vesicles

are small and round their outlines are clear, being preserved, no doubt, by the fixing of the pigment cells on the one hand, and of the deep staining matter, which usually forms their proximal walls, on the other (see figs. 17 and 18, where the shaded vesicles represent deeply stained walls). As soon, however, as they lengthen out, the walls become so delicate that they collapse under the violent processes of fixing, hardening, and preparing the sections. It is common to find in young eyes great empty spaces where the rod layer should be between the retina and the pigment, the spaces occasionally interrupted by single, short, thick, deeply staining rod-like structures, one here and there having survived. That elements of some form or other filled these gaps is absolutely certain; indeed, the ragged remains of membranes can often be seen fringing the distal ends of the nuclei, and protruding a little from the *membrana limitans externa*. A great many sections show nothing but this, and one is apt to become hopeless of ever seeing the vesicles which, in life, had been crowded together in those gaps. On one occasion I found one of these spaces occupied by a single large vesicle with a complete pigment cell, which had left the pigment epithelium, inside it. In time traces of long vesicles become more frequent because they are supported and preserved by being in contact with other more formed and stronger elements (Part I, Pl. 3, fig. 16). It is when a number of very fragile vesicles are mutually supporting and squeezing one another that they disappear from our sections leaving hardly a trace behind.

In sections of retinas killed at night I have succeeded at last in finding vesicles intact. They are slightly mottled and dotted over with stain, and I conclude that they owe their preservation largely to this fact, viz. that their walls were strengthened by this staining matter, as appears to have been the case with the rods and the cone tips in the retinas above referred to from Table Mountain. Fig. 12 shows a group which have fortunately been preserved intact, and fig. 13, *a—e*, are elements from the same retina.

On looking at these our attention is at once arrested by figs. *a—c*. We have apparently typical cones with their points thrust into terminal vesicles. A little reflection, however, shows how such appearances could be easily produced as transitory phases. To make it clear I give a diagrammatic series, fig. 14, *a—d*. *a* represents an unmodified vesicle protruded as a long oval. The pressure caused by the gradual protrusion of new vesicles will be exerted upon *a* in the direction of the arrows shown in *b*, with the result that *a* will take the form shown by *b* (cf. the middle vesicle, fig. 12). In the narrow neck of *b* staining matter accumulates. Continuation of the pressure further lengthens the neck, and at the same time the adding of new vesicles forces back the pigment cells.¹

In the stage *c* I have introduced a refractive globule, which we may assume to have come out of the distal vesicle as matter absorbed by it from the pigment, as explained at length in Part II. At this stage it is again the turn of the element whose form-changes we are following to receive another discharge from the retina or, as argued in Part II, from its nucleus. This discharge drives out the staining matter which occupied the neck, so that it protrudes into the distal vesicle. The three figures 13, *a—c*, show three distinct degrees of thrust, quite accidentally selected, the figures having been drawn in the order shown in the plate before I was at all clear as to their meaning. In *a*, only the narrow tip of the matter from the neck has been pushed into the sac; in *c*, the tip and a portion of the refractive globule, in this case the matter composing the tip itself has been disarranged against the distal end of the vesicle; in *b*, a larger portion of the staining matter still has been thrust outwards into the sac. These curious "cones,"

¹ This lengthening of the vesicles widens the distance between the pigment layer and the body of the retina. The width is greatest in the centre of the retina, and in very young eyes diminishes rapidly on either side. This is certainly due to the greater activity of vesicle formation, i. e. of rod-production in the area of most active functioning.

then, are due to the driving out of the staining matter which had accumulated in the neck of the squeezed-up vesicle. There is no telescoping of the membrane into itself. It is simply another form of the phenomenon shown by asterisks in fig. 9, where the original contents of what has hitherto been thought to be the tip of the cone become the proximal portion of the contents of the Schwalbe's rod, which arises as soon as the vesicle has assumed its cylindrical shape. The vesicle assumes this latter shape apparently in both cases as it becomes more and more turgid with matter received on the one hand from the retina, and on the other from absorption of pigment. The stages *c* and *d* in fig. 13 require no connecting links, *d* being the next stage produced by the filling up of the distal vesicle. Still younger and simpler stages of transformation of vesicles into rods are shown in fig. 15. They need no comment.

Many interesting details of observations in relation to this part of the subject might be added, but the task of dealing with the retina of the Amphibia alone threatens to lengthen out so greatly that only points necessary to a clear understanding of the essential morphology of the retinas dealt with can be mentioned.

How necessary it is to understand the minute details of rod-formation I need hardly insist, that is if we are to make any progress with our researches into the mechanics of vision, for the rods are the specific structures which constitute the retina the specific organ of this sense. Believing, as I do, that all structures are produced both phylogenetically and ontogenetically only in response to physiological needs, I feel confident that in a case like this where the rods are produced in situ, and only when required, their processes of formation must throw light upon the mechanics of their functional activities. Some further details relating to these activities will be found in the next part.

PART V.

On the Removal of the Absorbed Pigmentary Matter from the Rods: an Explanation of the "Müller's Fibres."

In Part II I described in detail a set of phenomena which found their simplest interpretation in the assumption that the protoplasmic vesicles, known as the rods, protruded by the retina against the pigmented epithelium, absorbed the pigmented granules, and at times also the cytoplasm of the pigment cells. I propose in this paper to describe another set of phenomena which indicate the way in which the rods are freed from the excess of matter thus absorbed.

My results differ somewhat widely from any hitherto published, and especially from those obtained by the now popular impregnation methods, and I ought, perhaps, to make some excuse for not testing those other methods myself. My answer, I fear, can only be an apology. I selected the purely comparative method deliberately as the only absolutely certain way of obtaining light on intricate morphological problems, but the method is slow and laborious, and I grudge the time necessary to become an adept in the use of others, the results of which have still to be interpreted.

The pigmented matter was, as we saw, absorbed through the walls of the outer limbs, and some of it found its way through the transverse membranes into the inner limbs, where it helped to form the bodies known as the ellipsoids.

Part of the absorbed matter, then, finds its way through the transverse membrane into the inner limb. Here, unless it can find some further method of escape, it must accumulate and cause the inner limb to swell. No such swelling of the inner limb takes place in the Amphibia, but it is a striking phenomenon in many fish. This is the explanation of their "giant cones" which are so startling when seen for the first time (see figs. 20, *b*, and 21).

Although the rods and cones in the fish are not our special subjects in this paper, it will be necessary to enter into a few details with regard to them. In the very young the elements are seen to be nearly all of uniform size, with apparently the same form-phases in their production as we described for the Amphibia, viz. (a) small cones, (b) gradually lengthening cones, (c) Schwalbe's rods, and (d) fully developed rods (fig. 20, *a*). These are the natural stages in the formation of new rods in the amphibian retina. But in the fish, after the earlier stages of growth have passed, we find a very striking change, which seems to begin somewhere near the central region¹ and spread gradually over a large part of the retina. The change is as follows:—The inner limbs of the earliest formed rods gradually swell, until, in large and presumably old fish, they may be of monstrous proportions. So that though, while growth is still going on, there may be room for a few more young cones to protrude or for a few more of the inner limbs of the Schwalbe's rods to shorten while the outer limbs lengthen, that time comes to an end, and the retinal elements, at least over the modified area, consist entirely of (a) rods with monstrous inner limbs, and (b) bunches of Schwalbe's rods. The thin thread-like inner limbs of the latter find their ways between the swollen inner limbs of the "giant cones," while their numerous cylindrical outer limbs fill up the spaces between the comparatively speaking small outer limbs of the "giant cones." Fig. 20, *a, b*, shows comparisons between the conditions of the elements in young and old eyes in the viviparous blenny. Fig. 22, *a, b, c*, shows different parts of the same retina of a young plaice, *a* being near the centre, where it has functioned most actively. Similar results might have been shown from my sections of trout and stickleback. I had no sections of young cod for comparison with fig. 21, from an old fish, but we may judge of the original thickness of the elements by those which persist as Schwalbe's rods, a few of which are shown.

¹ Without having exactly located the region, I believe it to be the postero-ventral half of the central region.

Here, then, we have the very phenomenon we anticipated in the event of the refractive matter which passed from the outer into the inner limbs not being able to escape from the latter, at least as fast as it accumulates. These inner limbs become swollen with refractive matter. That this is the true explanation of the "giant cones" is rendered clear by a study of large fish like the cod. While in some, especially smaller fish, the matter filling the inner limbs is often difficult to define, in the sections I possess of the retina of an old specimen of this fish ¹ the refractive matter is quite recognisable. It is often seen in round homogeneous pellets just inside the transverse membrane, and usually continued some way up the axis of the inner limb. Round the periphery the contents are more granular. Here and there, however, the whole inner limb is one smooth, bright, homogeneous mass. These smooth, round, refractive pellets, which seem to accumulate above the transverse membrane, may be compared with the refractive globules in the cones of the frog. The refractive matter here, as elsewhere, is deeply coloured by plasma stains, such as eosin, but easily gives up nuclear stains.

If further evidence were wanted that the material which swells the inner limb is the refractive matter absorbed from the pigment granules by the outer limbs, it is supplied by those cases in which the colour of the absorbed matter is the same as that of the pigment. Such cases may be purely individual differences, and depend upon chemical variations in the pigments, or, perhaps, may be due simply to a too rapid absorption. Certain it is that though the shape of the pigment granules is lost, the colour of the absorbed matter may now and then be hardly altered. Among my sections of the plaice,² for instance, there is one in which the strong reddish colour of the pigment only slowly vanishes. It pervades all the outer half of the swollen inner limbs, sometimes extending some way up the "rod fibres." Other more striking instances

¹ Fixed with corrosive sublimate.

² Specially fixed and preserved for me by my lamented friend Mr. Martin Woodward at the Plymouth Marine Laboratory.

of the persistence of the colour right into the retina will be given below.

Before continuing to consider whether and how the matter can escape from these inner limbs, one or two points may be noted in passing. (1) The "giant cones" of the eyes of fish are not the morphological equivalents of the cones in the eyes of the frog. The latter are almost the earliest stages of rod formation; the former are not only fully developed rods, but, at least so far as growth proportions go, the most highly developed elements known in vertebrate eyes; their great size will have some bearing upon the question as to the length of the life of individual retinal elements when that question comes to be put.

(2) A very large proportion of these "giant cones" are double. Although the dividing line in the swollen inner limb may become very faint, and sometimes only traceable in tangential sections, the presence of two nuclei and of two outer limbs will always show whether any large "cone" is double. I have already shown that the peculiarities of the double cones in the frog are due to the fact that two protrusions of the retina start side by side almost simultaneously, and their forms are due to mutual pressure, both being subject at the same time to the general pressure which we have to assume to account for the ordinary cone phases of rod formation. In these great double "giant cones" we merely have two rods very close together, and about the same age. The fusion of their inner limbs will take place sooner or later, as these inner limbs swell with matter. The only part of the phenomenon which requires investigating is why these rods of similar age should be so frequently in pairs, or, tracing it a stage further back, why it is that as new nuclei arrive to send out their protrusions to form new elements for the growing retina, they leave so many pairs of nuclei between which they do not or cannot force their way.

(3) The remarkable change which takes place in the forms of the elements of the growing fish retina, from an

early stage with (a) small cones, (b) "Schwalbe's rods," and (c) fully formed rods, into a stage with only two kinds of elements, viz. (a) rods with enormously swollen inner limbs, and (b) "Schwalbe's rods" with long thread-like inner limbs, fully justifies the appeal which throughout all these papers we have made to pressure in order to account for the form-phases of the elements of the bacillary layer.

(4) The fact that in the eyes of all Vertebrates higher than fish refractive matter no longer accumulates in the inner limbs, at least so as to swell them to such disproportionate sizes, apparently justifies the conclusion that these accumulations are not helpful to the specific function of the retina.

Returning to the subject in hand, we must now show how the refractive matter ultimately escapes from these swollen inner limbs of fish retinas.

Reference to the sections of the cod leaves no doubt on this point; the very size of the "giant cones," and the coarseness of their connections, reveal what the smaller elements of other eyes could not so plainly show, at least until the facts have already been made clear. What I take to be a thick stream ascends from each of these "giant cones," and ends in a refractive clump against the outer reticular layer, the "cone" nuclei being sometimes elongated in the line of the stream. The terminal clumps form, as it were, conical expansions where the streams meet the tangentially arranged tissue of the outer reticular layer. Here, again, microscopic examination of the "stream," and especially of its large conical expansion, as seen in the cod, show at once the presence of the same matter as that in the inner limbs. It is not meant, of course, that this matter is alone present, for in what follows it will be seen that this refractive matter follows the threads and fibres of the cytoplasmic network of the retina. In this case the stream and its conical expansion doubtless have a cytoplasmic framework. These streams with their expansions occur in one form or another in most if not all eyes, at least as physiological stages, and are usually described as the "cone fibres" with

their intra-retinal terminal swellings.¹ Fig. 21 shows them in the cod, fig. 20, *a*, *b*, in the blenny, fig. 22, *a*, *b*, *c*, in the plaice, fig. 24, *a*, in the trout. These swellings, which are also specially conspicuous in Ramon y Cajal's figures from metal impregnation preparations, have hitherto found no explanation. They can now be accounted for as the points where the refractive matter escaping from the rods is temporarily arrested as it reaches the outer reticular layer. Confirmatory evidence can be seen in the fact that their size depends upon the functional activity of the part. Fig. 22, *a*, *b*, and *c*, shows three parts of the same retina. They show, as do all the fish eyes I have examined, that the retina is very unequally used up. The part *a* from the central region shows the largest swelling of the inner limbs of the knobs of the rod fibres, and the most marked using up of the inner and middle nuclear layers.

Having brought the refractive matter thus far in the eyes of the fish, we may go back and consider some other eyes in which it escapes from the outer limbs without swelling the inner limbs to such monstrous proportions. We can only refer to two cases, for a full treatment of the subject would require a close comparative study of the retinas of all the animal groups other than fish. The two cases chosen are especially interesting because they present such striking contrasts: (1) the Amphibia, with the inner limbs of their adult rods quite small and insignificant as compared with the outer limbs; (2) the Primates, with their long, rather thick inner limbs and thin outer limbs not much, if at all, longer than the inner limbs (see fig. 31, *a*).

(1) That the refractive matter escapes into the inner limbs in the Amphibia we know from the invariable presence of the ellipsoid. But the ellipsoid does not, as a rule, seem to grow, so that if refractive matter is always exuding through the transverse membrane, it must as rapidly be transformed and conveyed away through the retina. Certain it is that

¹ I need only refer to the familiar text-book diagrams, such as fig. 52, p. 46, of 'Quain's Anatomy,' 10th edition, vol. iii, part 3.

the inner limb is never swollen up with refractive matter. This sparing of the inner limbs in the Amphibia may perhaps be correlated with the enormous size of the outer limbs, for, so far as I know, no other group of animals has them so large in proportion. In the frog they are immense cylindrical vesicles, sometimes as much as 60μ long and 9 to 10μ in diameter. These, then, form very capacious reservoirs for the absorbed refractive matter, and, perhaps, seldom require, during any single period of activity, to overflow into the inner limb. In this way the matter may be dealt with by the outer limb itself, and, apart from the ellipsoid, escape directly into the retina along the wall of the inner limb without entering it. That the refractive matter escapes directly from the outer limbs into the retina along the walls of the inner limbs can sometimes be actually seen (fig. 25, *a*, *b*, *c*). These cases are all from the South African tadpoles referred to in Part III, which, owing to the immense quantity and dark colour of the pigment, are very instructive in this connection.

(2) Equally decisive for our contention are my sections of a human retina (the healthy normal eye having been excised for a morbid growth on the eyelid).¹ This eye had clearly not been much exposed to light before excision. We consequently find the outer limbs of the rods free from all refractive matter, and, like the inner limbs, almost clear vesicles but for the longitudinal fibrils and the granules taking nuclear stain. The fibrils on the inner limbs are dotted like those of the outer limbs in the Amphibia (see Part II and figures). Naturally no thick refractive streams can be seen running up into the retina from these rods. In very strong contrast with this are fig. 31, *a*, *b*, from retinas of the South African chama baboon,² which live in the full glare of the

¹ Kindly fixed in Perenyi's fluid and preserved for me by the well-known ophthalmic surgeon, Mr. E. Treacher Collins.

² They were generously obtained specially for the purposes of these researches by Mr. J. C. Rous, Tafelberg Station, Cape Colony, through the kind intervention of our mutual friends, Mr. and Mrs. Mallinson, of the Hex River Valley.

South African sun. Here the rods, and especially the large inner limbs, are mostly full of pigment, which can be seen streaming inwards into the retina, no longer forming single fibrils with terminal knobs, but great tangles of refractive matter, which, in the eye (31, *a*) with dark blackish pigment are dull and blackish, but in the eye (31, *b*) with bright yellowish-brown pigment are bright yellow-brown. I may say that after seeing how the pigmented matter streamed through the retina in the tadpoles brought from the slopes of Table Mountain, I was quite prepared to find something of the kind in the retina of the baboons, but was myself surprised to see how very obvious the escape of the absorbed pigmented matter into the retina is in these cases. The pigment is so dense that the colouring matter is not bleached in the rods, nor, indeed, does it undergo much loss of colour throughout its passage through the retina, as it usually does, say, in our indigenous *Amphibia*.

We have so far, then, traced the matter absorbed by the rods into the retina as far as the region known as the outer reticular layer. This is in many respects one of the most difficult parts of the retina to understand. The matted and deeply pigmented strands just below this layer in the baboon's eye, as well as the conical expansions of the ordinary "rod fibres," indicate that the absorbed pigmented matter is temporarily stopped by it. But the exact cause of the stoppage at this point I have not succeeded in unravelling. Krause thought that there was a tangential membrane at this place, his "*membrana fenestrata*,"¹ and certainly the first time one sees the outermost layer of nuclei of the middle layer arranged tangentially in a compact row, as shown in figs. 20, *a*, and 22, *c*, it is difficult not to think that Krause was right; but a study of older eyes (figs. 20, *b*, 21, and 24, *a*), or even of the more used-up parts of younger eyes (fig. 22, *a*), will show that these nuclei do not belong to any fixed morphological structure in the retina such as a membrane, but

¹ I have unfortunately never seen a copy of the book written by Krause under this title.

that they are merely nuclei of the middle layer passing outwards to become rod nuclei, and apparently flattened against the same tangentially arranged cytoplasmic tissue as that which detains the refractive matter in the manner described above. But the difficulty is not quite so simple as this, viz. that the stoppage of the nuclei going outwards is due to the presence of tangentially arranged tissue, or even to a mutual blocking of the way on the part of the nuclei moving outwards and of pigmented matter moving inwards. That this latter is not the cause is clear, because we find the same stoppage of the refractive matter even when, as in old eyes, nearly all the middle nuclei have passed outwards (see fig. 21). That other subtler complications are present can be gathered from the fact that the "rod fibres" often expand so as to form chambers in the outer reticular layer, and clumps of matter, often taking nuclear stains, may be seen in various conditions within these chambers. The relations of these clumps of staining matter to the terminal expansions of the "rod fibres" is not easy to ascertain; it is clearly necessary to keep them distinct in our minds. Borysiekewitz, who, I believe, is the first to figure these chambers,¹ took them for a new and hitherto undiscovered layer of cells, the "nuclei of which may sometimes be seen dividing." This description, however, does not apply to any eye I have yet examined, for I have found them in all stages of formation, sometimes in patches, sometimes all along the retina (cf. figs. 20, 22, and 24). A comparative study has convinced me that they are, as stated, merely expansions of the inner ends of the "rod fibres" round some peculiar mass of staining matter. Similar masses occur in the cytoplasmic chambers between the rod nuclei and the outer reticular layer in the frog, as shown in fig. 25, *b* and *d*. I can regard them, therefore, only as form-phases expressive of some physiological activity, the significance of which, so far as I have been able to unravel it, will be explained in a later paper.

But whatever is the real structure of the outer reticular

¹ Figured in 1887, but only claimed as a new "cell" layer in 1894.

layer, we shall see from what follows that the refractive matter sooner or later finds its way through it. We shall, indeed, now proceed to show what very startling effects its passage may have on the remaining layers.

Every sagittal section of a functional retina will show us the matter streaming through the middle nuclear layer and through the inner reticular layer, in which latter, however, the streams frequently lose themselves. Indeed, as must be apparent by this time to every student of the retina, I am putting an entirely new interpretation upon a very familiar phenomenon, viz. the "Müller's fibres." These, as is well known, have hitherto always been regarded as sustentacular, and are said to be formed out of distinct cells with recognisable nuclei. But a survey of many eyes and of eyes of the same kind at different ages, and of the same eye at different parts and in different physiological conditions, shows beyond all mistake that they are only streams of absorbed pigmentary matter finding its way through the retina. The current doctrine that they are sustentacular has been based solely upon their appearances when most developed. Well-developed streams may be found at almost any age, inasmuch as their development depends upon the degree of functional activity of the retina; but according to my experience they are found in this condition most frequently in very old eyes, as we shall see in detail below.

This, then, is the next point we have to demonstrate; the chief difficulty in the way of doing so is how to select from the abundance of the evidence only that which is the most conclusive.

First of all, it is best at the outset to record the observation that the refractive matter seems to be temporarily arrested by all cytoplasmic strands and membranes which are arranged tangentially, and only to form definite streams along strands arranged radially. Hence the rapidity with which the refractive matter passes through the retina depends upon the number of suitably disposed radial strands. From the rods to the outer reticular layer most of the strands are

radial, e. g. "the rod and cone fibres," hence accumulations of amorphous matter seldom take place in this layer. On reaching the outer reticular layer there occurs, as described, some temporary obstruction, the exact nature of which we have not attempted here to unravel. Through this reticular layer, however, the matter escapes. In young eyes with a plentiful cytoplasmic reticulum supporting the rows of nuclei, radial strands can be found in abundance to carry the matter through the middle layer to the inner reticular layer; but in older eyes, when the nuclei of the middle layer have been largely used up and the cytoplasmic reticulum is so reduced that but few radially disposed strands can be found, the refractive matter tends to accumulate often in large quantities (*m.n.* of the figures). In fig. 20, *b* (blenny), it is seen in small irregular patches; in fig. 24, *a* (trout), in thick tangential strands just above the outer reticular layer; in others, again, in immense tangentially arranged sheets. In fig. 23, *a* and *b* (plaice), the accumulations are near the outer reticular layer; in fig. 21 (cod) near the inner reticular layer. Many more figures might have been given, but these must suffice. Fig. 23, *a*, which was from a very large old plaice,¹ should be compared with fig. 22, *a*, *b*, *c*, which are from a young plaice, six inches long. In the least used-up part of the retina (*c*) no traces of these accumulations can be seen; in *b* they are beginning; in *a* they are already of considerable size, but in the very old fish they are enormous, and occur over most of the retinae. Of the few traces which I have so far seen of accumulations of matter in the retinae of mammals one is shown in fig. 30, where a thick strand runs along on the inner side of the outer reticular layer of a mouse which had been exposed to the light of an arc lamp. As it tapered away it gave off typical "Müller's fibres" in the way figured (see also p. 37 and fig. 26).

Before going on to the inner reticular layer, one word as to the supposed nuclei of the "Müller's fibres." These are nothing

¹ Specially selected for these researches by my friend the late Mr. Martin Woodward, while temporarily associated with the Irish Fisheries.

but the ordinary nuclei of the middle layer, and are used up like the rest. The appearances which have led to the supposition that they are nuclei of fixed morphological strands are due to the fact that single nuclei are not infrequently involved in these streams of matter, and, indeed, may at times apparently enter into some intimate physiological association with them. They may often be seen drawn out, and even at times robbed of their chromatic substance (see fig. 25, *c*). That they are not the nuclei of preformed sustentacular fibres follows from the fact that a comparative study shows that no such preformed structures exist, and that the so-called "Müller's fibres" are mere expressions of functional activity, and great numbers, even when best developed, have no such involved nuclei (fig. 32, *a*).

Coming to the inner reticular layer, this also, like the middle nuclear layer, undergoes changes with age (cf. *i.r.*, figs. 20, *a* and *b*, and 24, *a* and *b*). In very young eyes the reticulum is close, and forms what is called the "Punksubstanz." As soon as the eye begins to function, before which time there are no "Müller's fibres," streams of refractive matter begin to pass through it as very thin radial threads. Under a high power these are seen to be a fine zigzag; they are clearly not independent strands, but some staining matter running along the threads of the inner reticular layer. Further, they may branch or end suddenly in thin, tangentially arranged layers, from which new radial strands arise to run further in. Again, it is evident that these thin radial strands, which every one would at once call the "Müller's fibres," are not fixed structures, from the fact that in the retina of an older animal of the same kind (cf. figs. 20, *a* and *b*, 24, *a* and *b*)¹ they may have disappeared altogether, and instead there occur thicker streams finding their way in much coarser zigzags (fig. 21) along the strands and between the much more open

¹ The specimens of the viviparous blenny were fixed in Blés' fluid in the St. Andrews Marine Laboratory, and kindly given me by Mr. Wallace, who had prepared them for his own work. The trout were specially fixed for these researches by Dr. Kyle, also of St. Andrews.

meshes of the now altered reticulum. This fact is an absolute demonstration that these "Müller's fibres" are not independent preformed structures, but merely cytoplasmic threads of the retinal reticulum thickened with matter. When they run quite straight without any zigzag we must regard it as due to a gradually acquired radial rearrangement of the threads of the reticulum (cf. fig. 32, *a* and *b*).

Then, again, apparently at any point in the inner reticular layer, these strands may end suddenly, and the staining matter which was travelling along them may disperse to right and left (fig. 28, *c*). Many of the different aspects of the inner reticular layer are due to the presence of this refractive matter accumulated in different ways along its strands. One phenomenon is particularly suggestive; I refer to the darker zones which are frequently seen in it running for longer or shorter tracts round the retina. I have seen them frequently (see figs. 20, *a*, 22, *b*, *c*, 24, *a*). Borysiekewitz has also called attention to them. These dark zones are, as it were, waves of absorbed matter, records of former periods of functional activity, passing through the retina. This is not evident microscopically when the reticulum is a close "Punksubstanz," and the matter finely and evenly dispersed, but becomes quite obvious when the reticulum is coarse and open, for then the individual strands of the affected part can be seen specially thickened (see fig. 24, *a*).

All these facts become so obvious to any one who will take the trouble to study the retina comparatively that I feel it almost unnecessary to discuss the details any further. One or two points, however, remain to be noted. Just as the streams end almost anywhere in the inner reticular layer, the matter dispersing along the tangential strands, so fresh ones may begin anywhere within the same layer. And this brings us to the next layer, the nerve-fibre layer, or, as it is more commonly but less accurately called, the "ganglionic cell layer."

The appearance of the strands which run radially from the inner reticular layer to the membrana limitans interna is well known; they are the typical inner ends of the "Müller's fibres."

Usually comparatively thin as they leave the inner reticular layer, they expand into a conical arrangement of strands or membranes until they look in some sections like an arcade of expanding columns supporting the internal limiting membrane with its subjacent reticulum. Under the arches of this arcade are found the strands of the optic nerve, and the so-called "ganglionic cells." My faith in the sustentacular character of the "Müller's fibres" was first shaken by finding that in many of my preparations the majority of these columns arise from the edge of, or from various depths within the inner reticular layer itself, and that those which did so did not apparently differ from those which came through the inner reticular layer from the outer layers. It is quite apparent that when they arise from the edge of the inner reticular layer, they are in a position to collect and carry away matter from that layer (figs. 20, *b*, 21, 24, *a*, 26, *b*, 27). It is common also to find them arising in one of the darker zones above referred to, and when once the suggestion is made that they are, as it were, draining the inner reticular layer, a flood of light is thrown upon all their various shapes, for the typical arcade form I have described, though frequently found, is not invariable. In my preparations of the retina of a large cod, for instance, the typical expanding columns are somewhat rare, so that the matter, not carried away fast enough, clogs any strands or membranes running tangentially; see fig. 21, in which it coats the strands (*n.s.*) supporting the nerves.

Solid accumulations of this matter are, however, not often found in the nerve-fibre layer, although the clotting of the nerve and other strands which partly occupy the layer may be very dense (see fig. 28, *b*, from an old rat). Something more like solid accumulations are found in certain old eyes; e. g. figs. 26, *a*, *b*, and 28, *a*, show the absorbed matter accumulating within the conical expansions of the "Müller's fibres," sometimes causing them to change their forms and become nearly bell-shaped—the trumpet shapes shown in fig. 26, *b*, are apparently due to distortion of the sections.

But these accumulations and the clottings of strands and membranes are not sufficient to account for the lifelong streamings of refractive matter into this layer, and we should have to assume that it escaped finally through the internal limiting membrane to join the vitreous humour, even if the microscope did not clearly show us that this is what actually takes place.

In very few sections will the internal membrane be seen quite thin and clear; it is usually found thick and apparently laminated, and layers are frequently found flaking off into the hollow of the eye. That these flakes are, at any rate in part, due to the matter which comes along the "Müller's fibres" can be seen in the fact that in osmic acid preparations, in which these streams are usually blackened, the portions of the internal membrane which cover their conical expansions not infrequently show different degrees of blackening (see fig. 29, *a*). This shows that the refractive matter is certainly deposited on the internal limiting membrane. The question is, Does it pass through? It certainly passed into the retina through the external protoplasmic membrane, pushed out in the form of rods it traverses the whole thickness of the retina, and if it does not pass through the exactly similar protoplasmic membrane on the inside of the retina it ought to accumulate in large quantities. The only accumulations which we actually find in connection with this membrane are the above-mentioned laminæ, which, as is well known, belong to the vitreous humour. Some sections, indeed, show the stained "Müller's fibres," looking like so many processes rooting the similarly stained remains of the vitreous humour into the retina. And here let me say that absolute microscopic demonstration of subtle physiological processes may not be possible as so many separate details, but when all the facts are taken together the evidence may become as convincing as if we could prove each detail separately. This particular detail, however, namely, that the refractive matter absorbed by the rods passes ultimately into the vitreous humour, admits of demonstration.

This demonstration is afforded us by the fact that in the baboon's eyes the pigmented matter retains its colour right through the retina, being only slightly less bright and refractive near the internal limiting membrane, where it is present in enormous quantities. In the youngest baboon's retina the congealed vitreous humour was left in situ in the base of the retinal cup, and appears in the sections. Its layers nearest the retina are coloured like the pigmented matter on the retinal side of the internal limiting membrane.¹

Returning to our review of the passage of the matter absorbed by the rods through the retina, we have seen that if, instead of the matter having to travel along zigzag paths on the strands of the cytoplasmic reticulum, it found a sufficient number of radial strands running in continuous courses right through, the passage would be much simplified. All the accumulations of matter which we have described in the eyes of fish might be avoided. The most perfect radial strands which I have ever seen running through the inner reticular layer occur in sections of a human retina² which, from the scarcity of the nuclei in both the nerve-fibre layer and the middle nuclear layer, and from the condition of the inner reticular layer, I take to be that of an old individual (see fig. 32, *a*). It is hard to believe that such "Müller's fibres" as these were not permanent structural elements; if they were they had become so only during life, and to meet special functional requirements, for in the normal healthy retina of a man of forty-eight, referred to above, hardly a single straight radial strand can be found through the whole inner reticular layer. Faint zigzag streams alone occur here and there (fig. 32, *b*), but are not numerous. That there should be no pronounced "Müller's

¹ As this absorbed matter streams through all parts of the retina (except the blind spot), and during a lifetime of functioning, it is clearly a factor which no student of the vitreous humour can afford to ignore. It suggests, for instance, a new and very simple explanation of Stilling's canal.

² Purchased many years ago from Messrs. Watson, of Holborn, in a series of slides to illustrate the structure of the eye.

fibres," i. e. streams of matter passing through the inner reticular layer, in this eye, is just what we should expect, in view of the fact that for some days prior to excision it had not been exposed to light; but it is surprising that there should be no traces of any permanent rearrangement of the cytoplasmic reticulum so as to form continuous radial lines. It is possible that this only takes place in very old eyes, when both nuclei and cytoplasmic framework, all but its radial strands, seem to be disappearing (cf. the general condition of the inner reticular layer in fig. 32, *a*, with that in 32, *b*).

In the baboon's retinas, through which an enormous quantity of matter can be seen to have been passing, and in which the large inner limbs are filled with the same matter, all of it the same colour as the pigment, the conditions are as follows:—In the youngest retina (three months) thick yellowish-brown streams in immense numbers pass radially through the compact middle nuclear layer, but when they reach the inner reticular layer by far the greater number disappear; the few which seem to run straight through that layer, on examination with a high power, are seen to have a very zigzag and interrupted course. On the inner side of this layer dense streams again form and run towards the inner limiting membrane, expanding and losing their intensity before reaching it.

In this young baboon's eye, then, there are no clear radial arrangements of the fibres of the inner reticular layer which could, even under the most strained interpretation, be regarded as sustentacular.

In an adult male baboon the same is true, only the pigment is blackish. We again see what was described above for other retinas, that the reticulum of the inner reticular layer has become much coarser than in the younger eye, and consequently the zigzag of those streams which run continuously through is much more pronounced.

In an "old, very large male" the streams are still fewer in the inner reticular layer, apparently because every strand is clotted with pigmented matter, as is also every strand and

membrane between this layer and the internal limiting membrane where the dark brown of the pigmented matter is very dense. Individual streams can hardly be followed.

In these eyes, then, again, and in spite of the quantity of the pigment absorbed, we find the same difficulty as we found in the cod (cf. fig. 21, *i.r.*) in establishing direct radial paths for the escape of the absorbed matter through the cytoplasmic reticulum; that such paths do occur and may be very highly specialised we know from the (presumably old) human retina shown in fig. 32, *a*. These, seen alone, certainly appear as if they were sustentacular.

Other perfect radial tracks seem to occur normally in the Amphibia, for as soon as the eye begins to function, that is in quite young tadpoles, there arise distinct, smooth, nearly straight radial fibres through the inner reticular layer, and these become so tough in preservation that they can be isolated intact if a section is teased up or crushed on a slide.¹ Further, in tangential sections they often appear running through the inner reticular layer within a clear passage. It is possible that this clear passage may be delusive, and due to the fact that the adjacent parts of the reticulum are drained by them of any matter which would render their delicate cytoplasmic membranes or threads visible. Compare with these apparently clear courses of the "Müller's fibres" through the inner reticular layer, fig. 29, *b*, where "Müller's fibres" of a rabbit are shown cut transversely, and the tangential threads or membranes of the inner reticular layer thickened with matter are seen to be running into them.

But the important contrast comes later. The establishment of direct radial streams through the inner reticular layers in the young tadpole is quite natural, for we remember that in other eyes the nearest approach we found to a straight course was in young retinas (see fig. 24, *b*) when the inner reticular layer is a close "Punksubstanz." But whereas the

¹ Many of them with nuclei of the middle layer attached to them (see fig. 25, *c*).

streams in these fish retinas become more and more zigzag as the meshes of the inner reticular layer get larger and coarser, in the frog and toad, for some reason or other, the early straight paths appear to become fixed. Whether this can in any way be correlated with the other peculiarity pointed out in these amphibian retinas, viz. that the absorbed matter passes by, apparently without entering, the inner limbs of the rods, which consequently remain very small, we are not yet in a position to decide. It is, of course, quite possible that the physical condition of the absorbed matter not coming in contact with the staining matter in the inner limbs might be different, and consequently its action on the cytoplasmic framework of a retina might also be different.

It need hardly be pointed out that if a group of such streams as those shown in fig. 31, *a, b*, flowing through the layer of rod nuclei were to combine in or just after leaving the outer reticular layer (*o.r.*), and then flow on as one thick stream through the middle nuclear and the inner reticular layers, we should have the most developed type of "Müller's fibre," such as that shown in fig. 32, *a*. It is these most developed streams, looking as if they were permanent structural elements in the eye, which have alone been regarded as typical "Müller's fibres." Had all the minor forms of the same streams received equal notice, the error could never have been made of ascribing to them any fixed morphological significance. Such a wider survey would also have saved Borysiekiewitz, to whose works on the retina I should like here to express my indebtedness, from his conclusion that the "Müller's fibres" are tubes conveying the nerve-fibrils to the rod layer.¹ Only these most developed strands which seem to rise directly from the rods could possibly supply the necessary conditions, and, if this conclusion were correct, we ought to find such developed strands in all and throughout all the retinas of the whole of the Vertebrata. This, as we have seen, is very far from being the case. Equally mistaken, too, are the conclusions based upon the impregnation method. In Ramon y Cajal's

¹ 'Weitere Untersuchungen,' Leipzig und Wien, 1894.

well-known figures of "fixed morphological elements" revealed by the method, we find not only the "dendrites," but also the "Müller's fibres" in their most developed form, and the "rod fibres" with their terminal swellings all equally clearly shown. The interpretation which we have put upon the latter two makes it more than probable that a proportion at least of the "dendrites" are also nothing but the parts of streams already so frequently alluded to in the foregoing pages. I say a proportion of the "dendrites" for reasons which will be made clear in another paper, in which I shall also show that the nerve-paths through the retina can be demonstrated by ordinary methods of staining, and that they have no connection whatever with the "dendrites."

SUMMARY.

As the results so far attained in the preceding five parts of these studies are largely hidden under a mass of minute histological detail, it is better, at this stage, that a summary be given of the more important. In the next paper we shall deal with the question of the nerves, which naturally has a much wider bearing than any detail of retinal structure merely as such.

The conclusion which of all others now arrived at is of widest significance from a general point of view, is that the retina can no longer be regarded as built up of so many separate "cells," each with some definite and permanent morphological value. This view, which has always been taught hitherto, has recently to all appearance been strongly confirmed by means of the metal impregnation method. This appears to reveal several distinct types of cells mainly distinguishable by their positions and by the different forms assumed by the ramifications of their respective cytoplasm. It is now maintained, indeed, that these cells, to which special names have been given, have distinct and definite functions, so that if one

single one were absent, a blind spot would ensue as a necessary consequence.

The results here published, obtained solely by comparisons not only of different eyes but of the same eye at different ages, involve a direct contradiction to this interpretation of the phenomena. If there ever were distinct cells composing the retina, their walls were early lost.¹ The functional retina is a continuous cytoplasmic reticulum in which nuclei are suspended, and the nuclei are not stationary. (1) A large proportion of those which are present in the young retina move outwards when it begins to function to become the nuclei of the new rods required by growth. (2) Their places are supplied by others migrating inwards from the rim. (3) The outward movement continues as long as life lasts, for in old eyes the nuclei of both the innermost and the middle nuclear layers are found to have largely disappeared. Whether 3 is for the supply of new rods or for some regenerative process we have no means yet of deciding. These migrations, and especially this using up of the nuclei, in a retina which is all the while functioning normally, shows clearly that some other value must be assigned to its structural elements than that which is needed by the neuron theory as applied to this organ. It is clear that these nuclei are not the nuclei of cells taking part in fixed morphological chains, every link of which is essential. The nearest approach we obtain to anything like a permanent cell in the retina is the rod with its nucleus; that it would be inaccurate to persist in using the term "visual cell" in this connection will be conclusively shown in my next paper.

With reference to the retina itself as the specific organ of vision, by far the most important result obtained is the discovery of some new details relating to the origin and structure of the rods, that is of those structures which are peculiar to the retina as the visual organ. According to the

¹ What appears to be the gradual dissolution of cell walls may often be seen where the young retina is passing into the cells of the iris.

usual description they are of the nature of cuticular formations. This is a very natural summing up of the facts—(1) that they are almost certainly the end organs of the nerves, and (2) that their tips are filled with refractive matter of the nature of keratin. But the parallel with cuticular cells, although justifiable, is not very close. As protoplasmic vesicles thrust out against the pigment cells they absorb the pigment granules and (unless the quantity absorbed be too great, and its colour too intense) clarify them somewhat as the stratum lucidum of the epidermis receives and clarifies the pigment brought to it through the skin. Here, however, the parallel ceases, for while the cells of the cuticle perish with the waste matter they receive, and ultimately fall away as horn-cells, the rods get rid of their refractive contents, which stream away through the retina.

The working out of the finer structural details of the rods, taken up where the subject was left by Max Schultze thirty years ago, need not be repeated here, but one or two of the more important corrections of the current doctrine may be mentioned.

What are called the “cones” of the vertebrate eye, to which special functions distinct from those of the rods have been assigned, are not always analogous structures.

In the Amphibia they are the early stages in the formation of new rods, and their form-phases are due to the squeezing of new vesicles between the already existing rods.

In the fish analogous stages appear in very young eyes, but in older eyes the inner limbs of the earlier formed rods swell to such monstrous sizes that the conditions of the rod layer are altered, and the protrusion of new vesicles can no longer result in the formation of the same cone stages. The rods with the swollen inner limbs have been regarded as “giant cones,” although presenting no analogy whatever with the cones in the frog.

In the Primates, what are usually called the cones are, as in the fish, merely rods with swollen inner limbs. In the centre of clear vision, where the pigmentary matter is

absorbed in large quantities, all the elements are permanently of this character, but away from the centre only one here and there has its inner limb enlarged. Borysiekewitz refers this to the protrusion of the nucleus, but as the nucleus is not always protruded, I prefer to refer it to an extrusion of fluid from the retina. Not only does the early protrusion of fluid vesicles from the retina in the first stages of rod-formation make this probable, but also the fact that globules of fluid are continually escaping from the retina into the rods, as described and figured in Part II.

The striation of the rods, which has long been known, has now been traced to its true cause, viz. the existence of strands, sometimes taking stain, in the walls of the rod vesicles, while the lumina of these vesicles are occupied by a staining network in connection with these strands.

The refractive matter which fills the outer limbs of the rods is absorbed pigment, which is usually, but not always, clarified during the process of absorption. The correlation of this with the results of the classical researches of Boll, Kuhne, Ewald, and others I am not in a position to work out, for reasons given in Part V. It must be left to time, on the one hand, to show where we mutually confirm one another, and, on the other, to eliminate our respective mistakes. Had I commented on all the results obtained by previous workers whenever they overlapped the subject in hand, these papers would have been lengthened out indefinitely; as it is, the histological details given in them have had to be limited to a small selection of those available.

The curious zone formation within the retinal syncytium has been traced largely to the above-mentioned lateral movement of the nuclei of the middle layer from the rim towards the centre.

The "Müller's fibres," however startling they may appear at their highest development, are merely streams of the pigment matter which have been absorbed by the rods, and which, with many interesting variations of detail, pass inwards through the retina, eventually to join the vitreous humour.

EXPLANATION OF PLATES 3—5,

Illustrating Parts III, IV, and V of Mr. H. M. Bernard's paper on "Studies in the Retina."

N.B.—The measurements of the different eyes can only be approximate, because the shape is not always kept in very thin sections. It should be further noticed that sometimes a slightly older eye may be smaller than one obviously younger, a fact to be attributed to the accidents of nutrition.

In all the figures *m.l.* = limiting membrane, *g.l.* = "ganglionic cell" layer, *i.r.* = inner reticular layer, *m.n.* = middle nuclear layer, *o.r.* = outer reticular layer, *o.n.* = outer nuclear layer.

FIG. 1.—Frog tadpole (Perenyi's fluid). Eye diameter 0.32 mm. Part of section showing the spindle-shaped nuclei of the undifferentiated rim of the retina, attached to either the inner or the outer limiting membrane, and arranged on the axial side in curves bulging towards the axis of the eye. The arrow indicates the direction of the nuclear stream. A few of the nuclei already in the middle layer, selected because attached by trailing cytoplasm to the inner reticular layer.

FIG. 2.—Toad tadpole (Lindsay-Johnson's fluid). Eye diameter 0.528 mm. Part of section drawn with the camera lucida, to show the attachment of the inner reticular layer to the membrana limitans interna, this connection being apparently due to the nuclei trailing their cytoplasmic attachments behind them as they travel towards the axis of the eye. These nuclear attachments tend to accumulate on each side of the stream, but persist as an accumulation only on the inner side (see text, pp. 10—13).

FIG. 3.—Frog tadpole (picro-sulphuric and iron hæmatoxylin). Eye diameter 0.24 mm., to show a younger stage in the formation of the inner reticular layer. The nuclei, which in the central region are loosely arranged where this layer is beginning to form, are densely crowded, five to six deep, against the pigment, and are apparently pressing inwards from the undifferentiated rim where nuclear divisions (*k.*) are taking place. The arrows indicate the direction of the streaming; *o.r.* indicates a line of dark staining matter where the future outer reticular layer will run.

FIG. 4.—From the other eye of the same animal. In both these eyes yellow fluid was apparent in the pigment cells, and here and there also apparently in vesicles which appear among the pigment granules, and were probably protruded from the retina (cf. Fig. 18).

FIGS. 5 AND 6.—Frog tadpole (picro-sulphuric). Still younger eyes showing earlier stages in the formation of the inner reticular layer as a kind of splitting of the nuclear ranks into two divisions, those forming the larger division crowding outwards against the pigment, leaving a loose, matted, and staining reticulum in the space from which they have moved. In both sections it is noticeable that nuclei in the very centre have even gone from the innermost layer. In Fig. 6 the cornea (*c.*) is seen thinning and clearing of pigment over the axis of the eye, and a nuclear division (*k.*) is seen near the centre of the retina, the two facts together indicating that the eye was only just beginning to function.

FIG. 7.—Frog tadpole (picro-sulphuric). Eye diameter 0.20 mm. Shows a still younger stage (i.e. smaller, and with larger cavity in the lens). The crowding outwards of the nuclei in the optic axis not yet appreciable; the beginning of the split among the loosely arranged nuclei is, however, indicated by an accumulation of vacuolar reticulum along the line occupied later by the inner reticular layer; nuclei seem also to be breaking away from the innermost layer, that forming the later so-called "ganglionic cells." A slight curving of the lateral nuclei, like that shown in Fig. 1, is also seen. The yolk granules which obscure the section are not indicated either in this or in the last two figures.

FIG. 8.—Frog tadpole, from Table Mountain (Perenyi). Eye diameter 0.8 mm.; cross-sections of rods showing deeply stained internal reticulum; this changes its pattern when the focus is changed. The reticulum is sometimes forced to the sides by a refractive greyish mass, which at times may have a brownish centre of the same colour as the pigment. In these cases the reticulum frequently comes again into view on changing the focus.

FIG. 9.—From the same retina, showing the distal ends of the cones as vesicles, often torn, but nearly always leaving ragged proximal ends still attached to the conical tips of the staining portion; in other cases the vesicles are complete, and their distal ends are immersed in pigment; they are shrunken and often beaded with rows of dots. On comparing the elements marked with an asterisk and numbered 1 to 7, we can trace the transformation of a cone into a Schwalbe's rod.

FIG. 10.—The same, in which the relations are shown more completely. In both these figures the continuation of the vesicle membrane into that enveloping the "cone" is quite distinct. On the right is a new element with no staining proximal portion yet visible (cf. Part I, Pl. 3, fig. 2, *a*).

FIG. 11.—The same, showing the more direct transition of the "cone" into the rod, nearer the side of the retina where the elements are shorter (namely, 20 μ instead of 45 to 50 μ , as they are in Fig. 10). In this case the distal portion of the cone or new rod was visibly striated, which was not the case on the very young and still slightly swollen Schwalbe's rod shown in Fig. 9 on the extreme right.

FIG. 12.—From the same retina as Fig. 1. A group of elements in the

early vesicular stage, the large vesicles not destroyed by the reagents. The exact relations of the nuclei cannot be made out. The nuclei of the two youngest vesicles may, perhaps, be those shown in or on the outer reticular layer.

FIG. 13.—A few elements from the same, selected to show some of the form-changes from vesicles to rods. Most remarkable are the “cones” shown on the left (*a, b, c*). Their tips are quite clearly within vesicles. The phenomenon is explained in the next figure (14). The rod on the extreme right shows one of the bright staining globules referred to in Part II.

FIG. 14.—A diagram to explain the “cones” shown in Figs. 13 and 15, in which their tips are thrust into terminal vesicles. The pressure of new protrusions acting in the direction of the arrows converts the vesicle *a* into *b* and *c* with a progressively lengthening neck. The staining matter which accumulates in this neck (see the central element in Fig. 12) is then thrust outwards (1) by the outward movement of the nucleus, and (2) by a fresh discharge of material (*d*). These “cone” tips, therefore, have no other membrane than the vesicle into which they are thrust. They are therefore sometimes disintegrated, and without defined outline.

FIG. 15.—Frog tadpole (Perenyi). Eye diameter 0.4 mm. Still shorter and stouter elements showing the same phenomenon, taken at different distances from the centre of the retina. The formation of short thick rods out of vesicles can be easily understood.

FIG. 16.—From the retina of a frog tadpole (picro-sulphuric, safranin). Eye diameter 0.24 mm., showing early protrusion of vesicles against the pigment. Deeply staining yolk granules are shown here and there; they are left out of Figs. 5 and 6. Two nuclei are shown in the figure not yet in contact with the *membrana limitans externa*. They are selected because they show vacuoles inside. Intra-nuclear vacuoles and vacuoles extruded within the retina may be seen in all the sections of young eyes; the earliest phase of the inner reticular layer looks, indeed, like an aggregation of such intra-retinal vacuoles (cf. Fig. 7).

FIGS. 17 AND 18.—From the retina of another frog tadpole of about the same size (from 0.20 mm. to 0.25 mm.). Portions of sections differently magnified, showing more vigorous protrusion of vesicles against the pigment. Those quite in the pigment are often yellowish in colour, while those nearest to the nuclei are clear white, partially framed round with densely staining matter (iron-hæmatoxylin). In Fig. 18 the vesicles marked with asterisks were yellowish. In other slides the yellow fluid, which here appeared in vesicles, was certainly inside the pigment cells as well (cf. Fig. 4).

FIG. 19.—Frog tadpole (Perenyi). Eye diameter 0.8 mm.; a group of nuclei crowded outwards. The *membrana limitans externa* was not distinguishable; its probable position is indicated by the marks of interrogation. Where the crowding was seen, the nuclei of the middle nuclear layer was diminished from

four deep to only three (cf. the observation of Borysiewitz described in the text, p. 2).

FIG. 20.—From the viviparous blenny (Bles' fluid). *a*. Section of young retina, showing the early stage, with ordinary cones, Schwalbe's rods, and two adult rods forming a double rod. Note the layer of cells at *o.r.* and the dark zones in *i.r.* The nuclei *m.n.* were about eight deep. *b*. From old specimen of same. The rods in two kinds only—(1) double rods with enormous inner limbs ($22\ \mu$ by $14\ \mu$), "giant cones," and (2) Schwalbe's rods. The layer of nuclei at *o.r.* has disappeared, and most of those from *m.n.* The *i.r.* is coarse-meshed, and very few strands run radially through. *c*. From the same, to show the tight packing of the swollen inner limbs, or "giant cones."

FIG. 21.—Section of the retina of a very large old cod (corrosive sublimate). A "giant cone," with inner limb $42\ \mu$ by $22\ \mu$, and filled with refractive globules. Crowds of Schwalbe's rods packed in between the "giant cones." The rod fibres end in terminal conical expansions against the *o.r.* The nuclei (*m.n.*) are nearly all gone, while the *m.n.* layer itself is choked up with solid sheets of amorphous, finely granular matter, some of which is seen streaming away in a zigzag through the *i.r.* The nerve-strands (*n.s.*) are clotted with finely granular matter.

FIG. 22.—*a-c*. Section of the retina of a small plaice, 6" (Flemming), to show three parts of the same section, *a* being nearest the centre. To illustrate the gradual using up of the "ganglionic cells" (*gl.*) and of the nuclei of the middle layer (*m.n.*); the disappearance of the continuous layer at *o.r.* (the nuclei of Krause's "Membrana fenestrata"), and the increase in the rows of rod nuclei; the gradual accumulations of granular matter in the *m.n.*, and the swelling of the inner limbs.

FIG. 23.—*a, b*. From a very old plaice. *a*. Radial. *b*. Tangential, showing the enormous accumulation of amorphous matter, extending in this old fish almost completely round the retina.

FIG. 24.—*a*. Sections of retinas of Loch Leven trout, ca. 7" to 8" (Perenyi). *b*. Section of very young trout, var. ? To show the change of the *i.r.* from early "Punktsubstanz," with fine zigzag "Müller's fibres" running radially through it, to the coarse-meshed older condition, and with the threads thickly coated with matter in the outer zone. Similar thickened strands are seen just within the *o.r.*

FIG. 25.—From tadpoles from Table Mountain, and characterised by great abundance of pigment. The inner limbs remain small, and the "Müller's fibres" rise straight from the walls of the rods. In *b.d.e.*, just above the rod nuclei, are seen the dark-staining bodies which seem to correspond with those seen within the expansions of the "rod fibres" seen at *o.r.* in some of the foregoing figures, e. g. 20, *b*; 22, *a, b* (cf. text, p. 32).

FIG. 26.—*a, b*. From the retina of an old cat (13 years) (Flemming), to show the inner expanded ends of the "Müller's fibres," containing accumulations

of amorphous matter. In *b*, exactly similar bodies arise entirely from the inner edges of the *i.r.* The trumpet shape is due to accidental compression of the section.

FIG. 27.—From a very old dog, showing the same as Fig. 26, *b*, i. e. the inner expanded ends of the so-called “Müller’s fibres” arising entirely from the *i.r.* Matter obviously belonging to the vitreous humour is seen flaking off the membrana limitans interna.

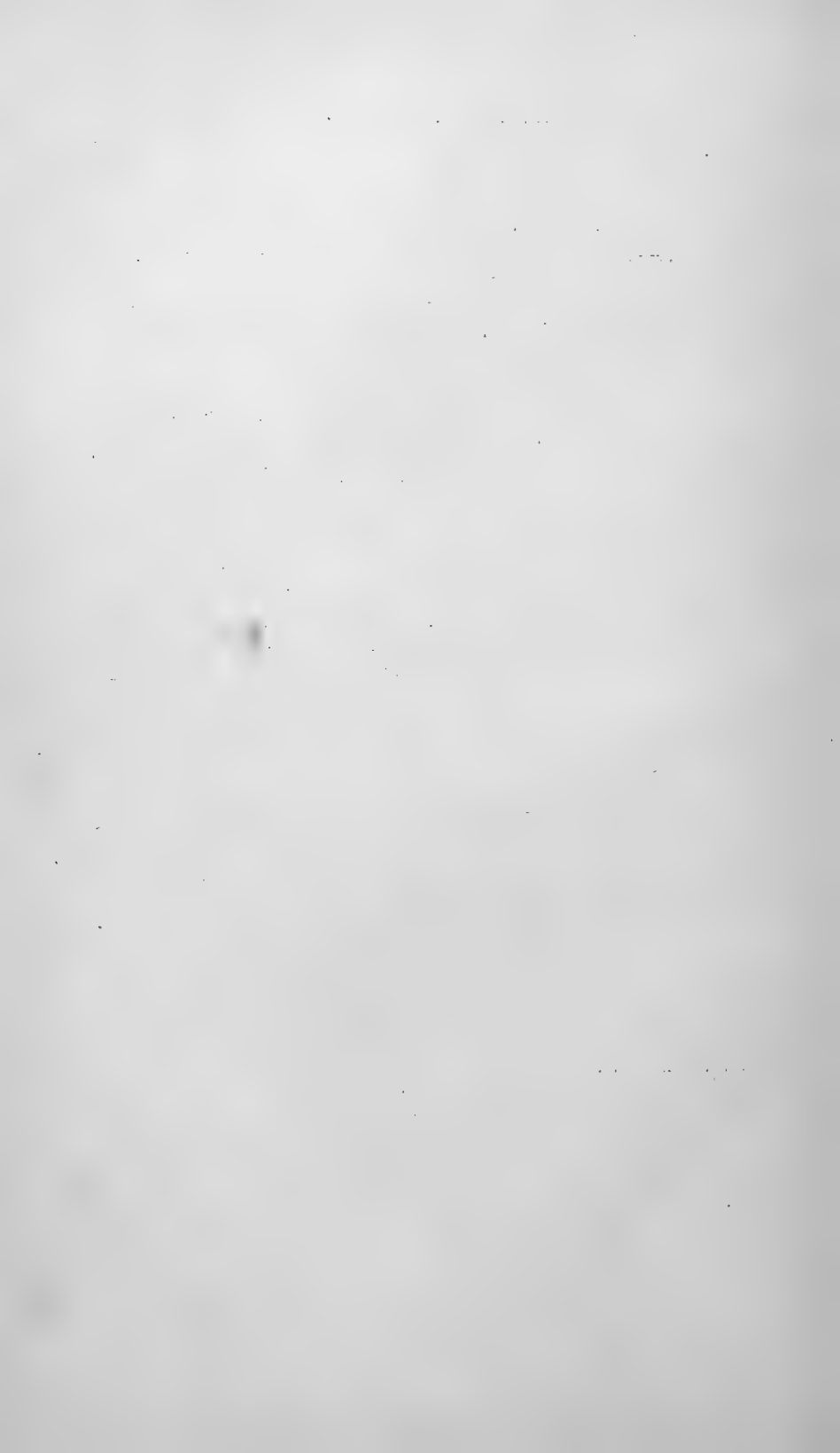
FIG. 28.—From a rat, 3 years old (Flemming). *a*. To show the flaking off of matter from the membrana limitans interna; the flakes show a delicate texture; the ends of the “Müller’s fibres” filled with amorphous matter, and rising from various depths in the *i.r.* *b*. To show the thickening of all strands and membranes indifferently, with matter coming from the *i.r.* *c* shows “Müller’s fibres” both losing themselves and starting again within the *i.r.*

FIG. 29.—From a rabbit (Hermann). *a*. From a tangential section, to show a portion of the membrana limitans interna divided into differently darkened areas representing the covers of the conical expansions of the “Müller’s fibres.” *b*. Two conical expansions with cross-sections showing the “fibres” as solid, and with the tangential strands of the *i.r.* running into them.

FIG. 30.—From a mouse which had been exposed to an electric arc lamp. A thick strand of amorphous matter winds its way through the cytoplasmic reticulum just inside the *o.r.*, eventually giving off, and bending up into typical “Müller’s fibres.”

FIG. 31.—From a chacma baboon. *a*. From an old male, with dense black pigment, which not only half fills the large inner limbs of the rods, but streams inward as far as *o.r.* without changing colour. *b*. From a young specimen, 3 months old, with yellowish-brown pigment, which also streams as far as the *o.r.* without changing its colour.

FIG. 32.—From the human retina. *a*. From a purchased preparation; apparently of an old retina, showing an enormous development of the “Müller’s fibres,” a very coarse inner reticular layer, and very few nuclei in the *m.n.* *b*. From a “normal healthy” retina of a man 48 years old, which had apparently been little used for some time prior to excision. Typical “Müller’s fibres” wholly wanting. Faint streams, often zigzag, and parts of streams alone occur, many of them processes from the so-called “ganglionic cells.” The *m.n.* was five nuclei deep; compare the number in *a*. But for this latter comparison to be of weight we ought to know how far from the centre of the retina the parts shown were severally taken.



Notes on the Relations of the Kidneys in *Haliotis tuberculata*, etc.

By

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With Plate 6.

NUMEROUS accounts of the structure and relations of the kidneys of Diotocard Gastropods have been written, chiefly by workers interested in the question of the homology of the Monotocard kidney, but in the various descriptions occur several contradictory statements, which naturally lessen the value of current theories on the subject. The present paper is the first-fruits of a study of the gastropod kidney, and it endeavours, by throwing new light on one or two disputed points, to help on the solution of this difficult problem. There are also brought forward certain suggestions concerning the kidney and reproductive organs of Monotocards which I feel convinced should be studied together; these suggestions are naturally extremely tentative, pending further work. In making this communication I wish to express my deep indebtedness to Professor Ainsworth Davis, whose advice and encouragement have alone made this research possible.

Haliotis possesses structures right and left of the pericardium (8), which, notwithstanding various views concerning their relations, are generally allowed to be the representatives of the right (7 R) and left (7 L) kidney of the primitive Dioto-

card. The structure on the left of the pericardium is a small sac, whose walls contain, in parts, lymphatic tissue; it is doubtfully renal in function, and is called the papillated sac. That on the right side is the functional kidney, and it possesses various large lobes, including a long anterior one (A. L.) stretching forward on the left flank of the great shell muscle. All workers also find two openings at the back of the mantle cavity: one (1) placed definitely on the left side of the rectum, the other (2) further towards the right.

Von Jhering (1), Perrier (6), and Wegmann (4) consider that the right opening (2) is the orifice of the functional kidney (7 R), and the left one (1) that of the reduced left kidney (7 L) or papillated sac. They state that the gonaduct (from 6 R) opens into the right kidney (7 R). Haller (9) found that the right kidney (7 R) communicated with the other (7 L), and that both opened by the orifice (1) of the left kidney, the right orifice (2) belonging solely to the gonaduct. My observations on these points agree with those of Perrier, who also worked with *Haliotis tuberculata*. Haller used *H. glabra*, and it would be necessary to study that species before rejecting his statements. Perrier, Wegmann, and Erlanger (8) described a pericardial communication for the papillated sac, but not for the functional kidney; while Haller found a funnel opening from the pericardium into the right kidney, but was unable to discover an internal orifice to the papillated sac. The former result has been generally accepted, and it is quoted as evidence that the right kidney is really degenerating in the *Rhipidoglossa*, and that the kidney surviving in *Monotocards* is therefore the *Diotocard* left (7 L). The methods adopted by these authors were the usual ones of dissection, injection, and section cutting, but in this case there seems to be another line of investigation. It is well known that the *Diotocard* female liberates her ova in an irregular fashion a few at a time into the mantle cavity. Now, if the gonaduct communicates with the kidney, ova might be found at a certain season in the cavity of the latter; and, further, if this kidney has a peri-

cardial opening stray ova might even make their way through it. It therefore seemed to me advisable to examine specimens of *Haliotis* taken during the breeding season.

Haliotis tuberculata may be obtained in fair numbers round the coasts of the Channel Islands, more especially on the rock-strewn shores of Guernsey and Sark, where it passes its sluggish life attached to the under-side of large boulders. It therefore lives with ventral surface uppermost, and is said by the fishermen often to die if removed from its attachment and left in the reverse position. It frequents the upper part of the Laminarian zone, and seems to feed largely on small algæ. The breeding season in this locality I have found to extend from about the end of December to the middle of February, and the specimens used for this investigation were collected in Guernsey during the spring tides of that period. They were soaked in 5 per cent. formalin, and mostly examined within a few days of their capture. The specimens were carefully taken out of their shells, and before they were placed in water or dissected at all their pericardia were opened on the left side well away from the kidney wall. The contents of the pericardial fluid were then examined, and found to consist mostly of corpuscles, a few epithelial cells, and sundries. One specimen, however, yielded a pinkish fluid, in which floated several ova; while two or three others also yielded each a few ova in the same way. The ova are very different in appearance from the other pericardial contents, and from the components of the various tissues abutting on the pericardium. They seem to retain their ovarian covering and a short stalk for a considerable time, very few having been found without them. The nucleus is prominent, and there is a small granular accumulation usually near the short stalk (see fig. 4, *b*).

An interesting feature in the female at the breeding time is a characteristic pink coloration more or less diffused over the whole body, but most noticeable on the covering of the hepatic cæcum, on the pericardial wall, on the head above the tentacles, and on the floor of the mantle cavity.

After sampling the fluid contents of the pericardium the contents of the right kidney were examined, and proved in several cases to be roughly divided into two sorts of material—darker brown fluid with excreta, and lighter coloured fluid containing both excreta and ova. Just behind the opening into it of the oviduct the kidney is partially subdivided by an internal projection of its right wall. The two parts are respectively an anterior one containing ova as well as excreta, and a posterior one containing almost solely the latter (see fig. 4).

From these finds of ova it seems justifiable to conclude—

a. That the gonaduct opens into the right kidney (6 R into 7 R).

b. That the right kidney has a pericardial pore (5).

c. That the anterior part of the right kidney is becoming connected more particularly with the reproductive system.

Injections of the right kidney were rather unsatisfactory, as might be expected considering its large size; but injection from the pericardium, on the other hand, showed very distinctly a pericardio-renal communication (5) near the anterior right-hand corner of the pericardial cavity (8).

By careful dissection of uninjected specimens from the pericardial side, an opening was found high up on the right wall of the pericardium, near its anterior right-hand corner. A fellow-student, Miss A. Ritchie, kindly confirmed this for me in another specimen. The opening as seen in dissection seemed fairly distinctly lipped, and is possibly imperfectly valvular; it is situated near the point where the duct-like portion of the kidney may be said to begin (5).

Further along toward the external opening, the wall of the functional kidney comes near that of the papillated sac; I have not, however, been able to find any interrenal communication such as Haller describes for *Haliotis glabra*.

Despite numerous attempts, by dissection, by examination of contents, and by injection, I have not been able to find evidence of a pericardial communication with the papillated sac, a conclusion in accord with that of Haller, but not with

that of Perrier and Erlanger. The wall of the sac is very thin, especially where it overlies the branchial vein. When it abuts on the pericardium it is also thin-walled over a part of the area, but the remainder is thickened into a mass of lymphatic tissue. There are vascular connections taking blood to the efferent branchial vein and thus to the auricle direct. Perhaps some one of these is what has been taken for a pericardial communication of the papillated sac.

To sum up, therefore, I think that *Haliotis tuberculata* has two separate kidneys right and left of the pericardium, opening externally by separate apertures (1 and 2). I find, also, that the gonaduct opens into the right kidney, which is the functional excretory organ, while the left kidney is partly degenerating into lymphatic tissue, and is becoming connected with the efferent branchial vein by direct blood-channels. So far my results agree with Perrier's. I find, further, in opposition to Perrier, and in agreement with Haller, that the right or functional kidney communicates with the pericardium (viâ 5), while the left one does not. The evidence adduced is, in part, of a different nature from that brought forward by the authors mentioned.

The foregoing results, if correct, lessen the divergence hitherto supposed to exist in this respect between *Haliotis* and *Patella*. The limpet has two kidneys right (7 R, fig. 3) and left of the rectum. The right kidney is very extensive, and performs most of the excretory work; it has several lobes, including a subrectal one (S. R. L.), which abuts on the wall of the pericardium. It serves as an exit channel for the reproductive elements, but evidence I have collected recently seems to hint at liberation of ova, at any rate, by rupture as well. This matter, however, needs further investigation.

The small left kidney is situated between the rectum and the pericardium, its circulatory system connects it intimately with the auricle. It is not shown in the diagram.

The right kidney communicates with the pericardium (8), the opening (2) being in the floor of the kidney's subrectal

lobe (s. r. l.), but opinion varies as to a pericardial pore of the left kidney, the latest statement being that of Mr. E. S. Goodrich (11), who makes certain he has found it.

Mr. Martin Woodward has recently published a valuable account (14) of *Pleurotomaria Beyrichii*, in which he says that the right (7 R) and left (7 L) kidneys of that animal are in many respects comparable with the corresponding parts in *Haliotis*. The efferent duct of the right kidney (7 R to 2) is prolonged forwards, and has thick glandular walls in the female, so that it is practically an oviduct. Woodward found a pericardial opening and canal (4) for the left kidney but not for the right, a result which, if confirmed, makes *Pleurotomaria* an exception to the general rule.

The most primitive type of kidney in Diotocards is, however, that of *Cemoria* described by Haller (9). Here both kidneys are well developed and functional, each communicating with the pericardium (via 4 and 5), and each receiving genital products from the gonad of its side. If this type is truly primitive we can, as Haller has said, derive from it the excretory organs of *Pleurotomaria*, *Haliotis*, and the *Trochidæ*, from which series the *Docoglossa* and the *Fissurellidæ* would be fairly early offshoots. Throughout, the left kidney and the left gonad degenerate, whilst the right kidney becomes both the functional excretory organ and the exit channel for the sex products. The right kidney retains, in most forms, its pericardial pore.

Perhaps the most marked contrast between the Diotocards and their Monotocard descendants is the presence of numerous accessory genital organs in the latter and their complete absence from the former group. In the latter, also, the reproductive and excretory systems are entirely separate. We must therefore seek out hints of the coming change among the ancestral forms.

Mr. Woodward has shown that the excretory duct of the right kidney of *Pleurotomaria* is practically transformed, in the female, into an oviduct (vide fig. 2).

In *Haliotis* the large anterior lobe (A. L., fig. 4) of the right

kidney is, practically, an accessory genital organ in posse. The external opening (2) of the right kidney is evidently becoming a genital pore.

Trochus and Turbo vary very much in this respect, but in some species the "anterior lobe" is very sharply marked off from the rest of the kidney. There is the same conflict of opinion between Perrier and Haller about the reno-pericardial funnels in Trochus as in Haliotis, and Haller finds also an interrenal communication in Trochus gibberosus. With regard to these reno-pericardial funnels it is noteworthy that in all Tænioglossa, even in the most primitive (figs. 6 and 8), and in Nerita there is a well-marked communication between kidney and pericardium on the right side of the latter—a fact which strongly supports the view here put forward, that the right reno-pericardial pore is retained, as a rule, in the Rhipidoglossa.

If the right excretory pore (2) becomes monopolised by the genital system, the functional kidney must find an exit for its excretory products; and it seems probable that this exit is through the external opening (1) of the left kidney, which would thus be the homologue of the Monotocard excretory aperture. The probability of this is increased by the fact that in no Monotocards have traces of a pore or sac been found to the left of the kidney opening. This view, however, entails the further supposition that the right kidney, or rather its posterior part, comes to communicate with the left kidney, and Haller claims, as was mentioned above, that such a communication already exists in Haliotis glabra and Trochus gibberosus. Perrier contradicts Haller, though he, too, supposes that the two kidneys come to communicate; he, however, almost certainly errs in stating that the Monotocard kidney opening is the right one (2) of Diotocards, for this statement raises a serious difficulty as to the homology of the genital opening.

Bouvier found such an interrenal communication in Ampullaria (5), and Perrier justifiably uses this observation in support of his views above mentioned. He further supports

his conclusions by observations on the "renal gland." He brings forward much evidence in favour of considering this gland, so generally found in *Tænioglossa*, as a modified vestige of the left kidney which has become intimately connected with the "pericardial gland." This gland consists of tubules which are lined by ciliated epithelium and open into the renal cavity (see fig. 9, *b*). The absence of this renal gland from the primitive *Paludina* suggests the hope that further work will reveal traces of the old left kidney in a less modified condition. The probability that further work will result in the discovery of the above-mentioned interrenal connection in other forms is increased by the fact that such connections are by no means unusual in Mollusca. They exist in several Lamellibranchs and in Cephalopods, and, without presuming to suggest that they are homologous throughout, their occurrence diminishes the improbability of their occurrence in Gastropods.

The remaining problem is the derivation of the accessory reproductive organs of the *Tænioglossa* and of their descendants the Opisthobranchs and Pulmonates.

The male has, typically, a large penis (*P*) at the right side of the head; this organ is retractile in more primitive forms (*Paludina*, fig. 8), but permanently extruded in more specialised forms (*Buccinum*, fig. 10). A very similar structure is found in the hermaphrodite Opisthobranchs, though their hermaphroditism has been shown by Pelseneer (10) to be due to the development of a male gonad in the female.

Mr. J. E. S. Moore (12) found an archaic form in Lake Tanganyika, which he named after its abode—*Tanganyikia rufofilosa*. The female of this animal possesses a brood pouch (B. P., fig. 5, *a*) on the left side of the head in the position of the penis of a male *Paludina*. He found the same structure in *Melania episcopalis*, and both also possessed a groove connecting this pouch with the genital aperture. This strongly resembles the spermatic grooves of some Opisthobranchs, and similar grooves also exist in the females of some *Tænioglossa* (figs. 8 and 9), while in the males the

spermatic duct (s. d.) running to the penis along the floor of the mantle cavity (fig. 10) or situated internally (fig. 8) has probably been formed by the covering in of such a groove. A groove of this kind is found in the male *Tanganyikia*, but the penis is absent (fig. 5, *b*).

From these facts Mr. Moore has argued that the common ancestor of *Tænioglossa* and *Opisthobranchs* possessed some accessory reproductive organ which had probably become separated from the genital duct and remained connected with the genital opening by means of a groove, which tended to become covered over. This accessory reproductive organ was somewhat variable, as it is lost in all *Tænioglossate* females except the two named, though the groove is retained in a few. In *Opisthobranchs*, which are originally female, and in male *Tænioglossa* this organ becomes the penis, while the groove is very often covered over and thus transformed into a duct. The development of such an accessory reproductive organ *de novo* is a difficulty further enhanced by the presence of the groove, but it is still premature, perhaps, even to suggest that possibly its ancestor is the anterior lobe of the *Diotocard* right kidney. It is interesting to note that *Typhobia horei* has a penis (fig. 7 *p*) which is extruded apparently *viâ* the genital aperture, and which is placed as an anterior dilatation on the reproductive duct.

To sum up, it will be most appropriate to give a brief statement of the views of previous workers and of the chief points raised in this paper.

The first theory is that of Professor Ray Lankester. He believed that the excretory aperture of *Monotocards* is the left kidney opening of *Diotocards*, but he thought also, from its position with regard to the rectum, that the *Monotocard* kidney (7) was the *Diotocard* left (7 *l*). Since these views were stated, the supposed absence of a pericardial pore of the right kidney has been used as evidence of the degeneracy of this organ, and, consequently, in favour of homologising the *Monotocard* kidney with the *Diotocard* left. Erlanger's work on the development of *Paludina* is also quoted in

support of this homology, but the structure which he takes to be the forecast of the Diotocard right kidney is merely a problematic and very transient vestige. The right kidney, on Lankester's view, would become part of the reproductive system.

Haller thought that the reproductive organs developed a duct which became continuous with the right kidney duct and opened through what was previously the right kidney aperture. The left kidney, he thought, degenerated, but became connected with the right, so that the Monotocard excretory organ was mainly right kidney, but opened through the left kidney's aperture. According to him, only the right kidney retained a pericardial pore.

Perrier agrees with Haller's conclusions except as regards the pericardial communication, which, he holds, persists only on the left side. He differs also in a point of great importance, for he says that the Monotocard kidney opening is the Diotocard right. His principal contribution to the discussion is the tracing of the fate of the left kidney. This, he found, became the renal gland, consisting of tubules lined by ciliated epithelium, and opening into the kidney cavity. This gland was, he said, typically associated with that ancient molluscan feature, the "pericardial gland." The conclusions supported in this paper are—

1. Lankester's view that the renal aperture of Monotocards is the left one of Diotocards. This opposes Perrier's conclusion.

2. Perrier's and Haller's view that the two kidneys in some Tænioglossate ancestors came to communicate inter se.

3. Lankester's and Haller's view that the right kidney opening becomes the genital aperture. This opposes Perrier's conclusion.

4. Haller's view that the right kidney retains its pericardial communication in most Diotocards. This opposes Perrier's conclusion. The evidence adduced is partly new.

5. Perrier's and Haller's view that the Monotocard kidney is composed of the right kidney of Diotocards, together with the cavity of the left (whose walls form the renal gland).

This I would slightly modify by stating that it is the posterior part of the Diotocard right kidney which seems to me to become the functional part of the Monotocard kidney.

6. I would also suggest, in a very tentative fashion, that perhaps the forecast of the accessory reproductive organ, which becomes penis or brood pouch in the Monotocards, was originally a dilatation on the reproductive duct. Perhaps even an earlier condition of this organ is what has been called the anterior lobe in the Diotocard kidney.

After outlining these conclusions I saw Mr. Martin Woodward's paper on *Pleurotomaria Beyrichii*. In a short discussion at the end of his paper he speaks in favour of conclusions identical with Nos. 1, 2, 3, and 5 above, but from his observations on *Pleurotomaria* he sides with Perrier as regards 4. Even, however, had I not found a reno-pericardial communication for the right kidney in *Haliotis*, I think there would still have been a balance of evidence from *Fissurella*, *Patella*, perhaps from *Trochus*, and especially from *Nerita*, *Nassopsis*, *Paludina*, etc., in favour of the view that the Monotocard reno-pericardial opening is that of the Diotocard right side.

More work on the *Trochidæ* and *Neritidæ* is necessary for any further advance towards certainty in the matters above discussed, but it is interesting to note that *Nerita* has a single kidney with a well-marked pericardial opening on the right side of the pericardium, while the external aperture is to the left of the rectum. The genital system, described by Haller, is quite separate from the excretory organ, and lies to the right of it. The kidney has no anterior lobe corresponding to that of *Haliotis*. The chief interest of the *Trochidæ*, etc., arises from the theory put forward by Perrier, Bouvier, and others that these forms are very near the ancestral stock of the Monotocards. Mr. Woodward, however, does not seem to share this opinion.

It has been a difficulty to represent, in diagrams 1—10 the true relations of the anal, excretory, and genital openings, and Professor Davis therefore suggested to me the addition

of schematic cross-sections of the mantle cavities of various forms, showing the rectum and oviduct or right kidney duct cut through, and the relative position of the left kidney or the Monotocard kidney opening.

It will be seen that the openings of right and left kidneys in Diotocards have the same positions, with reference to one another, as the genital and excretory apertures in Monotocards. The relation of these openings to the rectum, on the other hand, varies to some extent in different forms.

The most striking feature of these diagrams (11—15) is the migration of these openings to the animal's right side, and we also notice the disappearance of the right ctenidium and the folding over to the left side of the originally right leaf of the other ctenidium.

In the primitive Gastropods, as in Cephalopods, the incoming streams of water entered the gill cavity on either side and bathed the gills, after which they made their way out along the median line of the cavity, taking away the excreta from the openings on this line (fig. 11). Later on, the left kidney degenerated, being perhaps partly pressed out of existence, and the right kidney became the sole functional excretory organ. This process, already begun in *Haliotis*, was correlated with the disappearance of the ctenidium from the right side, and to the shifting of anus, renal, and reproductive openings to this side. The respiratory stream in such forms (figs. 12—15) would now come in along the left side and go out past the anus, etc., along the right, in-current and excurrent streams being thus freed from mutual interference. This more perfect separation, Professor Davis thinks, was the advantage which led to natural selection of variations along the lines of the changes just mentioned. This clockwise shifting of apertures and ducts has in some cases been continued so far that one or more have become situated along the extreme edge or even, in some cases, on the floor of the mantle cavity, so that their original relations appear, in horizontal plan, to have been reversed. *Paludina* shows a further modification, for here the renal opening is situated

between the anus and the genital aperture (fig. 15), but this peculiarity may be connected with the development of a ureter.

APPENDIX.

Since the above paper was written I have had an opportunity of seeing Pelseneer's recent publication '*Les Mollusques archaïques*,' and as he touches here and there the questions of kidney homologies, a short discussion of the work of this eminent scientist will add to the completeness of this little paper.

The chief new facts which he brings forward all support the conclusions I have ventured to set forth.

1. He finds that, in the Trochidæ, the right kidney has a communication with the pericardium, and, as I have found the same feature in *Haliotis*, arguments for the degeneracy of the right kidney of *Rhipidoglossa*, based on the absence of its pericardial communication, are definitely demolished. Pelseneer also finds the distinction between anterior and posterior regions of the right kidney of *Trochus* which has been mentioned in this paper as regards *Haliotis*.

2. He finds that Haller's interpretation of the kidneys of *Cemoria* was based upon errors of observation. As he has carefully examined specimens both from the source to which Haller had recourse (the Vettor Pisani collection) and from the White Sea, there seems to be little doubt that Pelseneer is right. If so, *Cemoria*, in its excretory and genital systems, resembles the typical Fissurellid. Its right kidney is of enormous extent, while the left is quite tiny, and only the right gonad is found.

This result is of great interest, as it is now possible to say that in all known *Rhipidoglossa* the left kidney is either extremely reduced or has undergone a transformation into a "papillary sac," an alteration which has profoundly affected its minute structure and its circulatory arrangements. It is therefore still more difficult than before to

homologise the left kidney of these forms with the single kidney of Monotocards.

Towards the end of his paper Pelseneer briefly discusses this question of the homology of the Monotocard kidney.

He first of all sets aside Haller's views, as every subsequent worker has differed from that writer regarding the relation of gonaduct and kidney. Proceeding next to discuss Perrier's theory, he points out the following weak points:

1. It necessitates the supposition that the rectum and right kidney have undergone relative translocation. This he finds difficult to imagine.

2. If we accept Perrier's further conclusion that the Monotocard nephridial gland is the remains of the papillary sac, we are forced to assume that in some type the kidneys came to communicate inter se. Having examined Ampullaria, Pelseneer denies that such a connection exists in that type.

Like the late Mr. Woodward, I cannot see that these objections are really vital. Perrier's theory of the nephridial gland seems to have much in favour of it, as also has the idea that while the Monotocard kidney is that of the Rhipidoglossan right side the opening is that which formerly belonged to the left kidney, but these are not essential to the theory. If, pending further evidence, we leave aside these additions, the whole of Pelseneer's second objection disappears.

As I have said further above, the frequent existence of the required interrenal communication in the more primitive molluscan classes seems to me to minimise the difficulty.

The question of translocation of the rectum is raised and discussed in rather a new light in this paper, and I venture to think that the conclusions reached very markedly diminish Pelseneer's objection. The rectum has undoubtedly shifted a great deal to the right, such shifting being far more important for the cleanliness of the ctenidium, and therefore for the efficiency of the branchial cavity, even than the rightward shift of the kidney.

On the whole it does not seem too much to say that, not-

withstanding the views he expresses, Pelseneer's results in this matter tend to strengthen the theory supported in this paper.

If this theory be adopted it is possible to say, now Pelseneer has settled the "Cemoria" difficulty, that in all *Anisopleura* the (post-torsional) right is par excellence the excretory side, even in those forms which still have a median anus. This opens up a possibility of very great and far-reaching interest as regards gastropod morphology.

Pelseneer's theory of the gastropod twist is now generally accepted as amended in the matter of terms by Amaudrut and others.

According to this the far-off untwisted ancestor had a gut going straight from front to back. This underwent—

1. A ventral flexure, giving the gut a cephalopod-like disposition.

2. A lateral torsion through 180° in a counter-clockwise direction, affecting all the animal except the head and foot. As a result of this torsion the branchial cavity and anus, previously postero-ventral, became antero-dorsal.

It is generally allowed that the pre-torsional position of the branchial cavity militated against its efficiency in a form possessing a creeping foot, for it would be pressed down against the top of the foot by the weight of the shell. Natural selection, therefore, led to the upward shifting of the cavity by survival of upward variations of its position. As far as I am aware, no one has yet shown how it is that the twist is counter-clockwise, and this has been an undoubted weakness in the theory; but the difficulty is, I think, removed by a consideration of the excretory organs and ctenidia on the lines suggested in this paper.

Let it be granted that in all Gastropods which have undergone the torsion the right is the excretory side of the branchial cavity, the left being more particularly devoted to respiration.

Then it is at least possible that this differentiation was already established during or before the torsion. It is not sufficient to argue that this is unlikely because the two

ctenidia of Cephalopods are equivalent, for the Cephalopods have increased the efficiency of this branchial cavity by a device of their own, which permits the retention of the kidney and anal openings in their ancient position. The early Gastropod had to adapt itself to a shore life, where the branchial cavity was not as easily rinsed as in the more pelagic ancestor, and where, therefore, the excretory products tended to spread over and interfere with the efficiency of the ctenidia, a tendency very imperfectly counteracted by the appearance of the slit in the shell. It was therefore desirable that any possible separation of incoming and outgoing currents should be encouraged. The reduction of its excretory function by the post-torsional left (pre-torsional right) kidney promoted this kind of separation between the incoming current of that side and the median outgoing one, and thus made the ctenidium of this side the more efficient.

Probably long before this the pre-torsional left side had become more especially connected with the genital function, for we find such a condition in practically all Cephalopods and Gastropods. As the genital products in the ancestral Gastropods were expelled through the kidney, the renal opening of this side was very important. This explains why it is that when one kidney diminishes it is that of the pre-torsional right (post-torsional left) side.

In such a form slight clockwise variations of the position of the branchial cavity would—

(a) Place the more efficient ctenidium in a less suitable position nearer the median line where the blockage of the foot would be most felt.

(b) Place this ctenidium at a lower level than the anus and excretory openings, and thus make it likely to get soiled and hampered by faecal and excretory matter falling on it, there being no such powerful outgoing clearing currents as in Cephalopods.

Counter-clockwise variations would, on the other hand—

(a) Place the more efficient ctenidium always in a better

position in the sense of being further out of the chink between foot and shell.

(b) Place this ctenidium at a higher level than the anus and excretory openings, and thus assure it against damage from the outgoing current.

It may be urged against the suggestion—

1. That, as Boutan says, we must not consider the excretory, etc., organs in discussing the torsion, because in ontogeny (in *Acmaea*) the torsion is completed before the definite appearance of kidney rudiments. The torsion, however, entails such a serious disturbance of organs that its appearance in ontogeny is peculiarly liable to be hastened, for the earlier it appears the smaller is the derangement.

2. That it proves too much; in other words, that according to it the pre-torsional left ctenidium should have disappeared before the torsion was complete. This ctenidium has disappeared in most Gastropods, but its occasional persistence is not a serious difficulty. In the first place its diminution would be retarded by the fact that its possessor was adapting itself to a life on the shore, where the time for breathing dissolved oxygen would be limited, thus making even a less efficient breathing organ temporarily valuable. In the second place this ctenidium persists mainly in forms which have evolved on special lines:

(a) Among the Fissurellidæ, where the deepening of the slit and further changes have shortened the path of the outgoing current, thus reducing the possibility of its interference with the incoming one.

(b) In the Haliotidæ, which have certainly come off from a very primitive prosobranch stock. Here, too, a secondary downward tilting of the other side of the branchial cavity has given this ctenidium an improved position.

(c) In modern Pleurotomariæ. The primitiveness of these forms is well known, and their very deep-water habitat seems likely to make respiration more difficult and so encourage the retention of all available respiratory tissue.

3. The other objection is that it accounts for little more

than the first 90° of the torsion. This objection is to some extent valid, but I think the process of completion of the torsion is correlated with the evolution of the shell-muscle, which I am at present endeavouring to investigate.

If the supposition that the pre-torsional left side performed most of the excretory function thus enables us to solve satisfactorily the mystery of the counter-clockwise torsion, this is surely a strong argument in its favour. I therefore venture to hold, even more strongly than before, the view supported in the earlier part of this paper, though it is not in harmony with the opinion of a zoologist of M. Pelseneer's eminence and insight.

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EXPLANATION OF PLATE 6,

Illustrating H. J. Fleure's paper "Notes on the Relations of the Kidneys in *Haliotis tuberculata*, etc."

EXPLANATION OF REFERENCE LETTERS, ETC., IN DIAGRAMS.

1. External aperture of left kidney of Diotocards and of kidney of Monotocards. 2. External aperture of right kidney of Diotocards and of genital system of Monotocards. 3. Anus. 4. Left reno-pericardial opening. 5. Right reno-pericardial opening. 6 L. Left gonad. 6 R. Right gonad. 7 L. Left kidney. 7 R. Right kidney. 7. Monotocard kidney. 7 N. Renal gland. 7 T. Tubules of renal gland. 8. Pericardium. A. L. Anterior lobe of right kidney. B. P. Brood pouch of *Tanganyikia rufofilosa*. P. Penis. Gr. Groove connecting genital aperture with brood pouch (sometimes vestige only). S. D. Spermatid duct on floor of mantle cavity, probably formed by covering in of groove. U. Ureter of *Paludina*. S. R. L. Subrectal lobe of right kidney of *Patella*. R. C. Renal cavity (Fig. 9, b). G. Rectum. LY. Tissue of pericardial gland, in which renal gland is embedded. ct. Ctenidium. ct^l. Ctenidium of right side in *Haliotis* (Fig. 11).

DIOTOCARDS.

FIG. 1.—Excretory and genital organs of *Cemoria noachina* (after Haller). Contradicted and disproved by Pelseneer.

FIG. 2.—Excretory and genital organs of *Pleurotomaria Beyrichii* (after Woodward).

FIG. 3.—The right kidney of *Patella vulgata* (after Lankester).

FIG. 4, a.—Kidneys, gonad, etc., of *Haliotis tuberculata*.

FIG. 4, b.—Ova of *Haliotis*, much magnified.

MONOTOCARDS.

FIG. 5, a.—Excretory and genital organs of the female of *Tanganyikia rufofilosa* (after Moore).

FIG. 5, *b*.—Excretory and genital organs of the male of *Tanganyikia rufofilosa* (after Moore).

FIG. 6.—The same organs in *Nassopsis nassa* (after Moore).

FIG. 7.—Male genital organs of *Typhobia horei* (after Moore).

FIG. 8, *a*.—Excretory and genital duct in *Paludina vivipara*—male.

FIG. 8, *b*.—The same—female.

FIG. 9, *a*.—The same—female of *Littorina litorea*.

FIG. 9, *b*.—Section of the hæmatic gland (pericardial gland and renal gland) of *Littorina* (after Perrier).

FIG. 10.—Excretory and genital ducts, etc., in *Buccinum undatum*.

SCHEMATIC CROSS-SECTIONS OF MANTLE CAVITIES.

FIG. 11.—*Haliotis*.

FIG. 12.—*Acmœa*.

FIG. 13.—*Trochus*.

FIG. 14.—*Littorina* female.

FIG. 15.—*Paludina* female.

In Figs. 5, 8, 9, *a*, and 10 it is supposed that the roof of the pallial chamber has been cut along near the middle line and reflexed.

In Figs. 11—15 it is not suggested that the various apertures, etc., occur in one and the same transverse section.

Notes on the Development of *Paludina vivipara*, with special reference to the Urinogenital Organs and Theories of Gasteropod Torsion.

By

Isabella M. Drummond.

With Plates 7—9.

THESE researches have been conducted in the Laboratory of Comparative Anatomy at Oxford, under the superintendence of Professor Weldon. They were originally undertaken in order to confirm or correct the account of the cœlom and its derivatives given by von Erlanger, and the results obtained are set forth in Part I of this paper. These then led to a renewed study of the whole course of development with a view to obtaining evidence upon the theories of torsion recently put forward by Pelseneer, Amaudrut, and Boutan. Von Erlanger's account deals only with the organogeny, and leaves the question of torsion on one side; it is perhaps for this reason that the development of *Paludina* has lately been sometimes regarded as too much modified and abbreviated to give any clear evidence on this point. Doubtless great modification has taken place owing to the loss of the free larval life; nevertheless, bearing this in mind, it is possible to a large extent so to disentangle the different processes of development which, owing to abbreviation, here go on side by side, as to be able to compare the results

obtained with those of the authors above mentioned. In Part II, therefore, I give a brief account of the development, aiming not so much at a description of the organogeny, except in one or two cases where my results differ from those of von Erlanger, as at making clear the changes in position and relative proportions of the organs in successive stages.

Before concluding this introductory note it is convenient to say a few words with regard to the plates. The outlines of the figures of whole embryos have all been drawn with a camera lucida from preparations of the whole animal, which is represented as transparent, the organs being shown by a dotted line. These have, in fact, been also traced, where possible, from whole preparations, but the tracings thus obtained have been added to after a careful study of sections. Figs. 11 to 17 explain themselves; they are for the most part transverse sections through the visceral hump, taken as far as possible through corresponding regions in successive stages, and all orientated the same way on the page, that is, as if the creeping sole of the foot were parallel with the bottom edge, in order to facilitate comparisons. All are taken looking from behind forwards,—that is, the left side of the figure is also the animal's left side.

Part I.—The Urinogenital Organs.

Von Erlanger (5 and 6), in his account of the developing coelom and its derivatives in *Paludina*, made known for the first time the existence of the rudimentary original left kidney, and showed conclusively that the existing kidney of the Prosobranchs corresponds to the definitive left kidney of other Gasteropods. Moreover, he brought the Prosobranchs much more closely into line with other Molluscs than had hitherto been the case, by describing the gonad as a derivative of the pericardium, and as discharging its products through a duct which was probably the duct belonging to the rudimentary kidney. While entirely agreeing to this extent with his results, I have arrived at conclusions with

regard to the manner of development which differ from those of von Erlanger in certain important points, and which bring *Paludina* even more closely into line with other Molluscs in respect of their cœlom.

According to von Erlanger, the pericardium, while still showing its two distinct chambers, forms two little evaginations, one on each side, which are the rudiments of a pair of kidneys. These, from the time of their first formation, lie against the ectoderm, which very soon forms the inward duct-like prolongations of the mantle cavity. Of these the right one coalesces with the original right kidney and forms its duct, while the left is arrested in its growth and the left kidney disappears. At a later stage a new outgrowth of the pericardium takes place in the same position as that which formed the original left kidney, becomes nipped off from the pericardial epithelium, and forms a little vesicle, which is the rudiment of the gonad. At the same time there is an ingrowth of the mantle cavity, which is presumably the arrested duct of the kidney that has disappeared. This grows towards the gonad, and finally fuses with it to form its duct. This account of the origin of the urino-genital organs has since been confirmed by the more recent researches of Tönniges (17).¹

I have nothing to add to von Erlanger's description of the early stages of development of the pericardium and kidneys, and of their relation to the mantle cavity. The series of somewhat oblique transverse sections of which one is represented in fig. 1 shows just such a condition as von Erlanger describes. In this figure the pericardium is shown with its two chambers still separated, the right being very much the larger of the two; the first rudiment of the heart, as appearing at *h.*; and just to the left of this is seen the original right kidney (*r. k.*) with its lumen, hardly showing in this section, communicating with the cavity of the pericardium. In a

¹ I have, unfortunately, not been able to obtain access to the original paper by Tönniges in the 'S.B. Ges. Bef. d. ges. Naturw., Marburg,' for 1899, and have had to rely upon the abstract in the 'Zool. Centralb.'

corresponding position on the wall of the narrow left-hand chamber of the pericardium is seen the little left kidney (*l. k.*), much less developed than its fellow on the right. In the next section of the series the solid ends of the two horns of the mantle cavity are found abutting each against the kidney of its respective side. It is probably at about this stage of development that von Erlanger describes the first appearance of the retrogressive development of the primitive left kidney. It is, indeed, extremely rudimentary at this time, and might easily be overlooked, but I cannot find that it ever wholly disappears; rather it might be said that its growth is arrested for a time, but at a slightly later stage it again resumes its development. Still less can I find traces of real retrogression in the primitive left horn of the mantle cavity. It ceases to grow, or at least does not grow nearly as rapidly as the primitive right horn which is to form the kidney duct, but it always retains its original relation to the pericardium, with its solid end abutting against the primitive left ventral corner, and is never, as von Erlanger both describes and figures, separated from it by a considerable space (see his pl. xxi, figs. 12 and 13).

As far as can be judged from the relative positions of the organs, the embryo from which his fig. 12, pl. xxi, is taken corresponds almost exactly to my fig. F in the drawings of the whole animal. A section across the visceral hump of an embryo of this stage is depicted in fig. 15, and shows how the little left kidney, far from having disappeared, as von Erlanger describes, is now larger than at the stage when he believed it to be most fully developed. This figure is wholly comparable with fig. 1, except for the change in the position of the organs due to torsion. The heart, now fully differentiated, is seen at *h.* in the same position relatively to the other organs as the similarly marked mass of cells in fig. 1, and the original right kidney, the definitive kidney of the adult, is cut across at *k.*, with its duct adjacent to it at *k. d.* The original left portion of the pericardium is even more narrow relatively to the right than is the case in fig. 1,

and in the extreme original left and ventral (now right and dorsal) corner of it is the left kidney (*l.k.*), contrasted with the right in being much less developed, but in every other respect perfectly comparable to it, and showing exactly the same relations to both the pericardium and the original left horn of the mantle cavity as it did before. Dorsally the pericardium is narrowed to a point beside the liver, and here a proliferation of cells is just beginning to take place, which is the rudiment of the gonad (*g.*).

The same structures are seen further advanced and more highly magnified in figs. 2, 3, and 4, which are three nearly adjacent transverse sections through a later stage. The position of the organs in the body is quite similar to that already seen, but here only the extreme (original) left-hand corner of the pericardium (*pc.*) is cut through. The rudimentary kidney is seen at *l.k.*, now showing a wide lumen, but having only a narrow communication with the pericardium, and the solid end of the duct is seen as before at *l.m.c.* Fig. 4 shows the rudiment of the gonad (*g.*), now a well-developed cord of cells, distinctly connected with the pericardial epithelium; while fig. 3, a section intermediate between figs. 2 and 4, just cuts through the edge of both kidney (*l.k.*) and gonad (*g.*), and shows their close proximity. This section is, however, chiefly interesting as showing the thickening of the cœlomic epithelium which connects these two organs, and very soon becomes indistinguishable from the gonad. A clear understanding of the position of these rudiments is important, and will readily be obtained by a comparison of the above-mentioned figures, especially of figs. 1 and 15. From these it will be seen that whereas the kidney is from the first on the original ventral side of the pericardium, the gonad is a dorsal proliferation, which from the time of its first formation lies close against the liver, the proximity of gonad and kidney being merely due to the extreme narrowness of the pericardium in this region. A comparison of these figures with von Erlanger's (fig. 5, pl. xxiii) seems to me to point to the conclusion that his

gonad (*g.*) is in reality the rudimentary left kidney, and that he has missed the true origin of the gonad altogether. I confess that I cannot fully understand this figure, but as far as I can make out, the gonad should lie in a direction at right angles to that in which it is shown, if it is to maintain the relation to the liver shown in fig. 6, and again in fig. 17, and this would bring it into about the right relation with the evagination of the pericardium marked *g.*, if we regard this latter as the rudimentary kidney. This view, moreover, would account for the discrepancy which exists between von Erlanger's account of the origin of the gonad as an evagination, and my own. The divergence in our descriptions of the duct is not so easy to explain, but I feel sure that von Erlanger is not correct when he speaks of the gonad (the left kidney, according to the present view) growing towards the ingrowth of the mantle cavity, for these are, and have been from the first, in the closest connection with each other (see figs. 2 and 15, *l. k.* and *l. m. c.*).

Fig. 5 shows a further development. The pericardium is cut across at the extreme right of the figure, from this the left kidney passes downwards at *l. k.*, and the communication with the pericardium is still shown at *r. p. c. ap.* On the left of the figure is seen the gonad (*g.*), still solid, but now fused with the wall of the kidney, so that the little connecting portion of thickened pericardial epithelium is no longer distinguishable. The duct is cut at *l. m. c.*, and does not yet open into the kidney. The exact position of these organs in the body may be seen in fig. 17, which is a drawing of a closely adjacent section of the same series. The lettering is identical, and the gonad, duct, and pericardium are all shown. It will be seen that essentially the same relations obtain as in earlier stages, the gonad following the liver, and keeping always on the inside of the coil.

From this stage onwards very rapid growth of the gonad takes place, so that it soon reaches the extreme tip of the visceral hump, and then it takes part in every coil as it is formed. At the same time it becomes hollowed out, from

the apex downwards, till its lumen is finally put into communication with that of the kidney, the opening being very close to the reno-pericardial aperture. A reconstruction of these organs from a series of transverse sections through an embryo, with a well-coiled visceral hump, is shown in fig. 6. The gonad (*g.*) is a hollow tube widening considerably at the apex, in reality following the coils of the visceral hump, but shown here spread out. The left kidney (*l. k.*) forms, as it were, merely the proximal extremity of the gonad (*g.*), from which it is separated at this time by no sharply marked histological differentiation. In this particular specimen the reno-pericardial aperture (*r. pc. ap.*) is retained even at this late stage, and I have occasionally found it in other embryos of about the same age; more often it appears to be closed, but it is difficult to tell for certain which is the normal condition, as the opening is small and might become artificially closed during preservation. In this case, however, the close proximity of the reno-pericardial and the reno-gonadial apertures is well seen. Even at this late stage there is as yet no communication between the left kidney and its duct, but the walls are now even more closely fused than before, and it is obvious where the exact point of communication will be. Details are shown in figs. 7, 8, and 9, which represent three sections through the same embryo from which the reconstruction was made. Fig. 7, taken across the line *a a* in fig. 6, shows the left kidney (*l. k.*) with its opening into the pericardium (*r. pc. ap.*), and its blind end lying against the duct (*l. m. c.*), and nearly opening into it. Fig. 8 is the next section, taken across the line *b b*, and again shows the new pericardial aperture. Finally, fig. 9 is a section across the widened extremity of the gonad at *c c*, showing the position in the narrow space between the liver and the outer epithelium of the body.

All the essential relations between the different parts of the genital apparatus are now established as in the adult, and I have not followed their development in later stages. It is interesting, however, in confirmation of the correctness

of this account, to notice von Erlanger's description of these organs when they have more nearly attained their adult condition, and are beginning to show the development of the actual genital cells. Such stages, he says, show "dass bei beiden Geschlechtern ein wenn auch kurzes Stück der Leitungswege der Geschlechtsprodukte aus der Keimdrüsenanlage selbst hervorgeht;" that is, this small region, apparently belonging to and originating from the genital organ itself, never, in either sex, gives rise to genital cells. It is, of course, situated just at the junction of the gonad and the duct, which, as he himself points out, "findet in der Gegend statt, wo der Verbindungskanal zwischen Herzbeutel und Nieren sich findet." Surely this must be the original left kidney, still distinguishable in the adult.

To sum up, then, the original left kidney and its duct do not, as von Erlanger believed, disappear. Their development is arrested for a time, but they are both clearly present at the time when the gonad is formed as a proliferation from the original left dorsal extremity of the pericardium, and from this time increase in importance. The gonad is for a long time solid, and is connected with the kidney by a thickening of the pericardial wall on the left side. At a later stage the gonad becomes hollowed out, and its lumen communicates with that of the original left kidney, presumably by means of the pericardial thickening, which must also have become hollowed out. The genital products therefore pass through the original left kidney, and are ejected by its duct.

The theoretical bearing of these conclusions is obvious, in that they show how, even in the adult of one of the most highly organised of the Rhipidoglossa, an unexpectedly primitive condition of the coelom and its derivatives still obtains. Zoologists have long been agreed that the ancestors of the Mollusca must have had paired gonads, which shed their products into the coelom, to be carried thence by the kidneys; that the coelom is now represented by the pericardium, and that, though great modification has taken

place, a remnant of the primitive condition is found in the frequent connection between gonad and kidney in existing forms.

While there is almost perfect agreement with regard to the general features of the anatomy of the primitive form, however, there is considerable divergence of opinion as to the course which evolution has followed, and consequently various interpretations are put upon the structures of existing forms, while hitherto embryology has been almost silent, and evidence has had to be almost entirely drawn from the field of comparative anatomy. Pelseneer (12 and 13) and Haller (10 and 11) are among the chief writers who deal with the *cœlom* and its derivatives among the Prosobranch Gasteropoda, and still uphold quite different views upon many points, though they seem agreed in maintaining that a gradual loosening of the connection between gonad and kidney has taken place throughout the group. While Pelseneer, however, only maintains that the point of communication tends to shift away from its primitive position by the reno-pericardial aperture nearer to the external opening of the ureter, as is the case in the Lamellibranchs, Haller regards the connection between gonad and kidney as altogether severed among the higher Rhipidoglossa, a portion of the *cœlom* becoming specialised as the gonaduct. Connected with this is the different view which these two authors take of the homology of the existing kidney. Thus Haller regards the functional kidney as in all cases the right one (after torsion). Among primitive forms (e. g. *Fissurella*) this keeps its connection with the gonad, while that on the left side of the body loses its connection with the gonad and kidney, and is fast disappearing. Further stages of evolution are shown by *Halotis*, *Trochus*, and *Paludina*, in all of which the left kidney has entirely disappeared, and the connection between the gonad and right kidney is lost, while the latter has more and more passed over to the left side of the body. Pelseneer, on the other hand, has demonstrated, though this is still denied by Haller, that in both

the Haliotidæ and Trochidæ a very small definitive left kidney is present, and that the large kidney is the definitive right, and still maintains its connection with the genital organ.

It might seem that von Erlanger (5 and 6, see also 7) had already sufficiently demonstrated from embryology that the homologies which Pelseneer believes to hold for Haliotis and Trochus are equally true for Paludina. To this, however, Haller (11) objects that in a highly organised form, such as Paludina, torsion is very likely abbreviated, and the organs may be formed in their definitive position. This view is, it seems to me, quite untenable from von Erlanger's description, while a further study of the development of this form shows even more clearly that a complete rotation of the organs through 180° actually takes place in the course of development,¹ and that the adult kidney arises on the right, and ends on the definitive left side of the body. I have, fortunately, been able to add further to this evidence by showing how the gonad still stands in close relation with the definitive right kidney, though this has altogether lost its excretory character, and that no such separate duct as Haller describes is ever formed. It seems, then, that there is every reason for believing that the definitive right kidney has persisted throughout the Prosobranchia as the genital duct, in some cases, as in Haliotis, performing also its renal functions, while in Paludina these latter are carried on altogether by the left kidney, the right functioning only as a gonaduct.

With regard to the manner of communication between the two organs, Pelseneer and Haller are also in disagreement. In the Docoglossa, at least, Haller describes a ventral cœlomic chamber through which the genital products must pass in order to reach the kidney; while Pelseneer regards this so-called cœlom as merely a portion of the kidney itself, the gonad being in direct communication with this latter, and altogether separated from the cœlom, which is only represented by the pericardium. Whether Haller believes in a

¹ For evidence upon this point, see Part II of this paper.

cœlomic connection between gonad and kidney in the primitive *Rhipidoglossa* similar to that which he describes for the *Docoglossa* is not very clear. Most writers, however, have described the gonad as having become separated from the cœlom altogether, and having acquired a new opening into the kidney. To this, and also to Pelseneer's view that this opening occurs nearer to the external aperture in the higher forms than in the lower, von Erlanger's description of the course of development in *Paludina* lent strong support. This, however, has completely failed, for the communication between gonad and kidney has been shown to be close to the reno-pericardial aperture in *Paludina*, as Pelseneer has described in *Fissurella* and other primitive forms, while traces of an original cœlomic connection between the two are found in the thickened ridge of pericardial epithelium described above, which can hardly be otherwise interpreted than as representing a groove in the cœlomic floor along which, in more primitive forms, the genital products passed to the reno-pericardial aperture. That the latter still remains open even after the communication between gonad and kidney is established is no real hindrance to such an interpretation, for the solid nature of the rudiments of both the gonad and the cœlomic connection shows that the ontogeny is abbreviated, and gives no exact picture of the phylogenetic events. The opening of this pericardial groove into the kidney must, it is true, represent at least a portion of the reno-pericardial aperture. Phylogenetically, we may believe, the edges of the groove drew together and a tube was formed, opening at one end into the gonad and at the other into pericardium and kidney at once through the reno-pericardial aperture. When, by abbreviation, this tube came to be formed in the course of development as a solid rudiment, it is easy to understand how the hollowing out and subsequent communication with the kidney might lead to the appearance of a rupture of the kidney wall. If this interpretation be correct, we have in *Paludina*, which has always been regarded as one of the most specialised of the *Rhipidoglossa*, a condition of

the urinogenital organs in every way comparable to that which obtains among the Amphinoma.

Finally, with regard to the origin of the single asymmetrical gonad in the Gasteropoda, both Pelseneer and Haller seem agreed that this has been formed by the fusion of the originally separate gonads of both sides. Phylogenetically, of course, this may have been the case, but ontogenetically there is no trace of it, the existing gonad being formed exclusively from the extreme left-hand corner of the original left division of the pericardium.

Summary.—The conclusions at which we have arrived are as follows:

(a) The embryology of *Paludina* demonstrates that the functional kidney of the adult belongs morphologically to the definitive left side of the body, as von Erlanger has already pointed out.

(b) The definitive right kidney is not lost, as von Erlanger describes, but persists as the genital duct.

(c) An indication of the original coelomic connection between gonad and kidney is found in the course of development of *Paludina* as a thickened ridge of pericardial epithelium, which finally becomes indistinguishable from the gonad, and, after it has acquired a lumen, communicates with the definitive left kidney close to the reno-pericardial aperture.

(d) The gonad arises as a solid proliferation of the morphologically dorsal wall of the pericardium. It arises from the original left side only, and shows no sign of a paired origin.

Part II.—The Development of *Paludina* viewed in Connection with Theories of Torsion.

(A) Description of Development.

Stage A (fig. 10).—The youngest stage which I have examined is a bilaterally symmetrical, oval embryo, with

well-developed velum, and already a slight swelling ventrally, which is the rudiment of the foot. The chief points in the anatomy are shown in fig. 10, which is a sagittal section through an embryo of this stage. The gut (*st.*) is a simple sac opening posteriorly by the anus (*a.*), and ending blindly anteriorly where it abuts against an insinking of the ectoderm (*m.*), the rudiment of the stomodæum. No very clear differentiation of parts is yet visible in the gut, but the ventral wall begins to show the vacuolated structure characteristic of the liver at a later stage. Dorsally to the stomodæal invagination the velum is seen cut twice (*v.*), and more posteriorly is seen the shell gland (*s.g.*), a deep sac, widely open to the exterior in other sections of the series. The mesoderm at this stage is represented simply by scattered cells.

Stage B (fig. B), shows considerable advance upon the last. The foot (*f.*) has grown out to form a prominent projection on the ventral surface. The shell gland is partially evaginated, and begins to form the visceral hump (*v.h.*), which, however, is still partly surrounded by a groove, deepest behind, and gradually disappearing anteriorly. The velar area has increased in size, and the tentacles (*t.*) are disappearing. The stomodæum has now broken through into the archenteron, and considerable differentiation has taken place in the latter. The middle portion has swollen and forms the stomach (*st.*), which lies at the apex of the visceral hump (*v.h.*), and from which the rectum runs downwards and backwards to open in the middle line behind (*a.*) The liver (*l.*) is an oval structure, sloping downwards and forwards from the apex of the visceral hump, where it communicates mostly with the stomach. The opening into the stomach is still so wide, and the demarcation between the two organs so vague in this region, that it is difficult to determine their exact relations, but the liver appears to lie to the left, and ventrally behind, while in ventral views of the whole embryo it can clearly be seen to pass below the œsophagus and to the right side anteriorly. It seems then to be an outgrowth of

the left ventral wall of the stomach. Below the rectum, and lying between it and the liver, is a little dense mass of mesoderm cells, which is just beginning to be hollowed out on either side to form the rudiment of the pericardium. Its position is shown in the figure at *p.c.* The otocysts have appeared on either side at *o.t.*, and are still widely open to the surface of the body.

Stage C (figs. C and C₁, and fig. 11).—Considerable growth in length has taken place, and the different regions of the body are clearly marked out. The foot is now a prominent organ in the anterior ventral region, and a slight constriction of the body separates the foot and head from the now well-developed visceral hump. This latter is surrounded posteriorly by the mantle folds (*m.f.*), which form a prominent ridge dorsally to the anus. At this stage the first rudiments of the mantle cavity appear as two little depressions lying one on either side of the anus. These are best seen in a ventral view of the whole animal, or in section. Fig. C₁ is a ventral view of a slightly older embryo than fig. C, but the essential relations of the organs are precisely similar. Here the two depressions are seen at *c.m.c.* and *r.m.c.*, the right one being considerably in advance of the left. The same depressions are seen in transverse section in fig. 11. Von Erlanger describes the first appearance of the mantle cavity as “eine kleine Grube” ventrally and just in front of the anus, but it is quite clear that at this stage there is a distinct rudiment on either side, the rectum passing down a ridge between them (*x* in fig. 11), to open directly on to the surface of the body. It is only at a later stage that the portion of the body immediately in front of the anus sinks in and unites the two original depressions, thereby including the anus within the mantle cavity. I have never been able to find a stage in which these two original depressions are symmetrical. If this stage closely corresponds, as I believe it does, with von Erlanger's fig. 1, plate xxi, he has overlooked an important point in the external anatomy of the embryo. It is not, as he says, perfectly symmetrical

externally at this stage, for not only is the symmetry disturbed by the inequality of the rudiments of the mantle cavity just noticed, but the whole visceral hump appears as if slightly tilted. The apex lies somewhat to the left of the vertical plane, which would divide the head and foot symmetrically, while the mantle fold on the left of the body is at a lower level from that on the right. This tilting is difficult to represent in surface views, though by rolling the whole embryo about it is perfectly easy to see. It is, however, sufficiently obvious in the transverse section through the hump (fig. 11), which is orientated on the page as it would be on the body, the line *aa* representing the vertical plane through head and foot.

In the internal organs there is little to add to von Erlanger's (5) account. The stomach and liver together form, as before, the apex of the visceral hump; they are now well-defined, though still retaining their wide communication with each other. Posteriorly the liver lies distinctly to the left, while further forward it gradually becomes almost ventral, passing over to the right, as before, in front of its opening into the stomach (fig. C_1). From the posterior end of the stomach the rectum runs almost vertically downwards to open in the position already noticed between the two rudiments of the mantle cavity. Just dorsal to these, and anterior to the rectum, are the two rudiments of the pericardium, still separated from each other, that on the right being a good deal the larger of the two, as we saw to be the case also with the mantle cavity (fig. C_1 , fig. 11). At the left, in fig. 11, a slight thickening of the pericardial epithelium is seen, which must be the rudiment of the left kidney; the right kidney is not yet formed. In the head region the first appearance of the radular sac is noticeable, and also the appearance of the ganglia of the central nervous system as thickenings of the ectoderm, as they have been already described by von Erlanger.

Stage D (fig. D, fig. 12).—The foot now begins to show for the first time a tendency towards the formation of the

characteristic creeping sole. The visceral hump is much developed and surrounded by a strongly marked mantle fold. Externally the most noticeable feature is a prominent bulge on the left side, which appears almost like the first formation of a coil (fig. 12). A transverse section across the visceral hump, shows, however, that it is simply a bulging out of the side of the body where the liver and stomach are located. A comparison of figs. 11 and 12, in fact, makes clear that this prominence on the left side corresponds to the original apex of the visceral hump, which has become still further tilted in the same manner as before, thus leading to a rearrangement of the organs when orientated with reference to the head and foot, while their mutual relations are retained. The liver now lies ventrally, as well as slightly to the left of the stomach.

Both liver and stomach are entirely on the animal's left, and the mantle cavity lies entirely on the right, while the anus, also, is displaced from the middle line and has travelled towards the right. Though readily seen by a comparison of transverse sections, this tilting is not so obvious when the embryo is examined entire, as it was at an earlier stage. This seems in part due to the great growth of the whole posterior region of the visceral hump, which has caused the rectum to bend forward to the anus, and the mantle cavity to take up such a position that the two original depressions lie almost vertically one above the other instead of almost horizontally, as in fig. 11, but chiefly is it the result of the rapid growth of the mantle downwards on the right-hand side of the animal's body, so that the right and left edges are now on about the same horizontal level, and a kind of false external symmetry is established in this respect. Rapid growth of the mantle leads, of course, to rapid extension of the mantle cavity, and consequently we find this far in advance of the preceding stage. The two original depressions are now united below the rectum, and form two horns which abut each against the kidney of its respective side, and then join and widen out to form the mantle cavity; a slight extension of the

mantle cavity to the right of the right horn is first noticeable in this stage, but will be more fully noticed in the next. The kidneys have been formed as outpushings of the (morphological) ventral wall of the pericardium on either side; the wall of the little left kidney and the adjoining wall of the left horn of the mantle cavity are cut through in the ventral part of the section in fig. 12 (*l. k.*), while the right horn is seen more dorsally at *r. m. c.* The ventral position of the left horn is also seen in fig. D at *l. m. c.*¹ The right division of the pericardium has become much enlarged, and now occupies a very considerable portion of the visceral hump, while the left division remains small, and lies in the narrow region between the liver and the left horn of the mantle cavity. The rectum bends sharply downwards from the stomach and then runs forward ventrally on the right to the anus, which lies just anteriorly to the junction of the two horns of the mantle cavity, and is now included in the latter.

Stage E (figs. E, E₁, E₂; figs. 13 and 14).—The foot has grown back into its definitive position, and is separated by a marked constriction from the head. The features of the visceral hump noticed in the last stage are now accentuated. The bulge on the left side has become much more prominent, and is a very characteristic feature, giving, even more than before, the appearance of sinistral coiling when looked at from above (fig. E₂). A comparison between figs. 12 and 13 shows that the essential relations are the same as in the last stage. The liver, now ventral to the stomach, has increased much in size; the pericardium, of which only a portion of the right-hand division is shown in the figures, has swollen, and is found extending for a considerable distance beside the stomach to the right of the original right kidney (see fig. E₁ and fig. 15); while close to the kidney on the right is found the first rudiment of the heart as a little solid ingrowth of mesoderm cells pushing the pericardial wall before it.

¹ For further description of the kidneys and their relation to the mantle cavity, see Part I of this paper.

Corresponding to this extension of the pericardium is a great development of the mantle cavity to the morphological right of the original right horn or kidney duct. This is shown in section with the pericardium lying above it in fig. 14, but is best seen in a view of the whole animal from the left, fig. E_1 ; here the left horn is seen ventrally at *l. m. c.*, the former roof of the mantle cavity is almost vertical and forms the posterior boundary, while the kidney duct (*k. d.*) is dorsal. Dorsal and anterior to this is now the chief extension of the mantle cavity, which is already visible in a dorsal view of the whole embryo (fig. E_2), and reaching behind to the mid-dorsal line, while it narrows slightly in front. Von Erlanger's description of an embryo of this stage differs considerably in respect of the mantle cavity from the above, but I am unable to reconcile his fig. 7 on pl. xxi with my own observations, for he both figures and describes (p. 358) the kidney duct as arising from the dorsal extremity which is now advancing over the mid-dorsal line, while a comparison of the series (of which two sections are shown in figs. 13 and 14) with the living animal (from which the outline of the mantle cavity in fig. E_1 was drawn) seems to me to show quite conclusively that the kidney duct arises in the position where I have marked it, and that the dorsal extension of the mantle cavity is a new development. If this be so, very rapid growth must have taken place in the region in front of a vertical line passing through the opening of the two kidney ducts, as is in fact the case, and this would lead to the increased ventral flexure which attains its maximum about this time.

Very little remains to be said of the other organs. The œsophagus is much lengthened, and has acquired a sharp downward bend before entering the stomach, which slopes obliquely upwards and backwards. The rectum bends sharply downwards and then runs forward to open on the right side in the mantle cavity. The right kidney has become further developed and forms a simple sac, still fairly widely open to the pericardium, while the left remains in much the same

condition as in the previous stage. The pericardial septum has disappeared.

Stage F (fig. F and fig. 15).—All the essential features may be seen in a view of the left side of the entire animal (fig. F). Comparing this with the similar view of the preceding stage we find that there has been very rapid growth in all parts of the body, especially in the "neck" region between the visceral hump and the head. The bulge containing the stomach and liver now lies nearly ventrally, the mantle cavity has extended over into the left side of the body, and just posterior to it is seen the pericardium, with the heart now well developed, and showing the auricle and ventricle separated from each other by a deep constriction. The rectum lies higher on the right side than in the last stage, and runs along the roof of the mantle cavity to open more anteriorly. Fig. 15 is a transverse section across the visceral hump of an embryo of this stage, and shows essentially the same relation of the organs to each other as in previous stages. The pericardium lies dorsally to the liver and stomach, and contains the heart. Dorsal to the pericardium are the two kidneys, the morphologically right, a well-developed but still simple sac, being seen at *k.*, its duct at *k. d.*; the opening of the kidney into the pericardium on the one hand and the duct on the other are neither of them shown in this section. To the right of the section is seen the rudimentary left kidney (*l. k.*), which seems to resume its development about this time. Its opening into the pericardium is well seen, and just above it is the solid end of the original left horn of the mantle cavity (*l. m. c.*). At the extreme right (morphologically left) of the pericardium the first rudiment of the gonad can just be distinguished at *g.*¹

A further important feature is the development of the visceral connectives, which are first visible at this stage. They arise anteriorly from the pleural ganglia and run back

¹ For further description of the gonad and its connection with the rudimentary original left kidney, see Part I of this paper.

on either side of the œsophagus to about the region where the anus opens, when they appear to lose themselves in the epithelium of the floor of the mantle cavity. The morphologically left connective is by far the stronger, and arises behind, just below the anus, while the morphologically right appears at the extremity of the right extension of the mantle cavity. The two connectives are thus separated by a considerable distance posteriorly, and they are not at present united by a commissure.

Stage G (fig. G and fig. 16).—The original apex of the visceral hump now points ventrally, though it is still more prominent on the left side than on the right, which gives the appearance, when the animal is looked at from above, of the visceral hump being set crookedly upon the foot. The œsophagus is elongated, and bends sharply downwards to open into the stomach. The stomach itself is much enlarged and lies chiefly ventrally, but ascends somewhat to open into the rectum, which then bends dorsalwards and runs forward in the roof of the mantle cavity to the anus. The mantle cavity now extends far down on the left side, especially posteriorly, and a portion of the kidney is visible in a view of the left side of the animal. The rudiments of the ctenidium are clearly formed as projections from the roof of the mantle cavity on the left. The kidney duct soon after leaving the kidney now passes below the rectum and runs backward on its right side to open into a sort of little pouch of the mantle cavity together with the genital duct, as the original left kidney duct may now be called. Von Erlanger's fig. 2, pl. xxii, shows very well the disposition of the kidney duct at this stage. The old relations of the rectum to the two original horns of the mantle cavity are thus disturbed, apparently by a drawing together of the edges of the mantle cavity in this region, the space between the two ducts being obliterated. The whole, or very nearly the whole, of the definitive mantle cavity seems, therefore, to be formed by the great extension of the original right horn, as noticed in Stage E. In other respects the mutual relations of peri-

cardium, kidneys, and ducts remain as in the last stage, but the gonad is now clearly formed as a thin cord of cells lying beside the liver.

The visceral connectives are now completely formed, and are united by a commissure, which in this stage lies asymmetrically, that is, wholly below the original right portion of the mantle cavity. This is, however, a secondary condition, and probably due to a tendency to place itself in relation with the symmetry of the external form, for a stage intermediate between this and the last shows that the commissure is formed in part from the floor of the mantle cavity just at the entrance of the genital duct.

Fig. 16 is a transverse section across the visceral hump in the region of the kidneys and heart. Liver and stomach lie ventrally, and above them is seen the pericardium, with the heart at the extreme left of the section. Dorsally to the pericardium is seen the kidney (*k.*) with its openings into pericardium (*pc.*) and duct (*k. d.*), both cut through. The kidney duct is beginning to pass ventrally to the rectum (*rec.*) as described above. The genital duct is cut through at *l. m. c.*, and the wall of the left kidney at *l. k.*

Stage H (figs. 17, 18, 19).—A definite coil is now being formed on the right side, about one complete turn of the spiral having been made, and the old crooked setting of the hump on the foot is nearly lost. In this stage the organs attain very nearly their adult condition in all essential points. The alimentary canal has increased much in length and become more coiled. The œsophagus bends down and towards the right to open into the stomach, which now stretches as a great sac below the liver, opening into it dorsally, and forming at the right-hand extremity a blind sac, which shows a tendency to follow the coiling of the liver (fig. 17). The rectum now opens out of the stomach quite ventrally and posteriorly, passes towards the left side of the body, then bends sharply upwards behind the pericardium, and runs dorsally along the roof of the mantle cavity, bending suddenly to the right just before it reaches the anus. The liver is greatly developed

and lobed, and forms almost the whole of the coil, being followed only to a very slight extent by the stomach and gonad. The pericardium is swollen, and the original left side, which has hitherto been so narrow, widens out considerably. In connection with this we may notice the advanced condition of the primitive left kidney and the now well-developed cord of cells (*g.*) which represents the gonad. The definitive kidney (*k.*) is seen on the left side of the section, and is easily recognisable by its slightly staining and now folded walls. The kidney duct (*k. d.*) is cut across to the right of the kidney, just where it passes below the rectum (*rec.*) as described in the last stage. Both kidney and genital ducts have now lost their primitive condition as simple specialised portions of the mantle cavity, and run forward in the roof of the latter as well-defined ducts, parallel to and to the right of the rectum, opening somewhat behind the anus.

The mantle cavity now extends very low on the left side, especially posteriorly, so that it is just cut in the section represented in fig. 17, the difference between anterior and posterior regions being much more accentuated than was formerly the case. The relations of the visceral connectives, as noticed already, though in a less degree in the last stage, are deeply affected by the asymmetrical growth of the mantle cavity, and as posteriorly the mantle cavity appears to lie wholly on the left side and with its floor almost vertical, so in the region just anterior to the commissure the two connectives lie almost in one vertical plane. A discussion of these relations may conveniently be left till the whole question of torsion is taken into consideration, but the twist of the connectives now remains to be described. As is well known, the two connectives are bilaterally symmetrical when they leave the pleural ganglia, almost immediately the right passes below, the left above the œsophagus, each to the opposite side of the body, after which they again resume their bilaterally symmetrical disposition, only that now on the right side is the original left, and that now on the left is

the original right. Following this twist in a series of transverse sections a peculiar relation between the œsophagus and the connectives is noticeable. In the region of the anterior ganglia and for some distance behind them the œsophagus is compressed so as to render its outline oblong in section. At first bilaterally symmetrical, it soon becomes completely asymmetrical, the long axis, as seen in section, sloping at first upwards and to the left, then passing through the horizontal position to slope upwards and to the right; in other words, the œsophagus apparently follows the connectives in their twist, as shown in figs. 18 and 19. At the completion of the twist the œsophagus becomes round in section, and passes back for some considerable distance lying between the two connectives, which are now once more in the same horizontal plane; then the connectives appear to become partly twisted a second time to take up the position with regard to the mantle cavity posteriorly, which has been already described. Unfortunately, in this region the œsophagus is still circular in section, and we have no direct evidence as to whether or not it follows the connectives in the same way that it does more anteriorly.

The relations between the connectives and œsophagus are seen to a greater or less extent in stages previous to this, but the description of them has been deferred till now as being casier of comprehension when they are present in such a marked degree. Already in Stage C, where we first noticed the tipping of the visceral hump, a slight apparent twist of the œsophagus was visible; this was more marked in the following stage, and increased in each succeeding stage up to the present. It is as well to notice that this condition of the œsophagus was plainly visible before the visceral connectives appeared at all.

It has already been said that the animal has now attained in all essential particulars to the anatomy of the adult. A few points, however, remain to be noticed, being for the most part only further developments of processes already begun. Of these the most obvious is the coiling which takes

place rapidly from this time. The tendency already seen in the stomach to grow out into a third sac which follows the coiling of the liver becomes considerably accentuated, while the gonad grows very rapidly and soon passes right up to the tip of the last coil. At the same time it loses its solid character, and, becoming hollowed out, acquires an opening into the duct, as described in the special part of this paper. Meanwhile the mantle cavity deepens, and the rectum grows forward to open near the anterior edge. It is during this growth that it acquires the characteristic disposition of the adult, passing from mid-dorsally behind, obliquely downwards and to the right, a disposition which is doubtless connected with the sharp bend towards the right described in Stage II. The pericardium alone begins at this late stage to show new relations, for it widens and becomes very irregular in shape, spreading amongst the other organs of the body so as to form a kind of general body-cavity.

Monstrosities.—Whilst collecting material for the study of the normal course of development a few monstrosities were found which presented some remarkable features. Although it seems impossible fully to understand the meaning of all the abnormal conditions found in these embryos, some of them seem to me to be of sufficient interest to justify the insertion here of a description of the main features of their organisation.

1. The simplest of these abnormalities is a small embryo between stages C and D in degree of development, perfectly normal in every respect, but wholly reversed. The liver and stomach form a bulge on the right side, while the mantle cavity and rectum are on the left, and all the other organs correspond in every particular. This is, so far as I know, the first record of a normally dextral Prosobranch so organised. It is unfortunately too young to show definitely the manner of coiling.

2. This embryo, shown in fig. M 11, is very remarkable. A camera tracing was made while it was still alive, and the organs put in partly from life and partly after preservation.

It was then sectioned in a plane transverse to the long axis of the visceral hump, and portions of these sections are portrayed in figs. 20, 21, and 22. The most noticeable feature is the greatly developed visceral hump, which was held erect over its head, bending in a decided manner at the apex, as though forming the first turn of an exogastric coil. A further remarkable feature is the perfect bilateral symmetry of the whole embryo, though it is obviously at an advanced stage of development, for though it is impossible to compare it with any given stage in the normal course, the head is well developed, the foot has found its normal creeping sole, and a small operculum is already present. The general disposition of the organs can be made out from the drawings of the whole animal. The stomach, it will be seen, forms the apex of the visceral hump, while just below it an enormously developed pericardium fills up for some distance the space between the descending œsophagus and rectum. Paired kidneys are seen at *k* and *k'*, but are better described in connection with the transverse sections. The same is the case for the great bulge in the lower posterior region of the visceral hump behind the rectum, which might be taken for the mantle cavity. Sections, however, show it to be merely the continuation of a great space which surrounds all the organs nearer the apex of the visceral hump, as seen in fig. 20, which is a transverse section in the region of the kidneys, and just below the pericardium. The mantle cavity is shown in figs. 20 and 21 as a narrow and symmetrical organ lying anterior (morphologically dorsal) to this great space, and apparently compressed by it. Towards the apex of the hump it forms two symmetrical horns which run back on either side towards the kidneys, but never fuse with them. Here a wholly inexplicable condition obtains. The pericardium, as already mentioned, is greatly developed, and it is not surprising, in an embryo otherwise symmetrical, to find out that here also two symmetrical evaginations have been formed. These, which must be the kidneys, are, on the one hand, very widely open to the pericardium, while on the

other they come nearly into contact with the horns of the mantle cavity, but no communication is formed. Instead, each kidney communicates with a sort of little vesicle, as seen in fig. 20, which at the same time shows the close approximation of the horn of the mantle cavity to the kidney on either side. Below the kidneys, that is, nearer to the anus, these two vesicles unite, and run back for a short distance as a single duct, which opens into the mantle cavity mid-dorsally, as shown in fig. 21. Shortly afterwards the anus opens on the same mid-dorsal ridge.

One further feature remains to be noticed, namely, the absence of liver. The stomach is well developed, but the only trace to be found of anything which might be interpreted as liver is a pair of little outgrowths of the alimentary canal just in the region where the stomach and œsophagus unite. These are shown in fig. 22. If these may be so interpreted, then the liver shares also in the symmetry shown by the mantle cavity and kidneys. The visceral connectives, as would be expected, run back perfectly symmetrically beside the œsophagus.

3. Other monstrosities occurred, but of less interest than the above. They all showed traces of symmetry in a greater or less degree, and some of them the same tendency to exogastric coiling, but in most cases the organs were deformed, and very much less clear of interpretation. Only one other, therefore, is shown here (fig. M III). This one is remarkable in that a greater degree of symmetry than is usual is combined with a slight sinistral torsion, and a fairly well-marked development of the mantle cavity to the left of the original left horn, as seen in the figure. The symmetry of the body is, however, confused, in that the pericardium is more developed on the right side than on the left. This embryo is further remarkable in that it is the only one of the monstrosities possessed of a clearly defined liver, which even in this case is very small, and hangs like a sac from the stomach ventrally, and slightly on the right side of the body.

(B) Theoretical Considerations.

I do not mean to attempt to give here anything like a complete historical summary of the many views which have been held on the subject of Gasteropod torsion and asymmetry. This has already been done more or less fully many times (see especially Simroth [16] and Boutan [2]), and I shall therefore confine myself merely to a very brief consideration of those views which lend themselves to criticism from an embryological standpoint, and upon which a study of the development even of a single form may throw some light. Such theories, therefore, as that put forward by Lang, which claims only to be phylogenetic, and for which confirmation is not sought from the facts of ontogeny, will be passed over altogether; while theories of authors who, like Bütschli, seek to base their conclusions to a greater or less extent upon embryology may be of some interest in this connection, and will therefore be considered. At the same time it must be remembered that the remarks upon these theories are professedly based only on embryology, and need not necessarily invalidate their phylogenetic value, though they may weaken the author's argument.

For the sake of convenience the theories under consideration may be placed in two classes. In the first of these are placed those theories which maintain that the present condition of the Prosobranchia has been brought about by a simple process of unequal growth, resulting in the forward movement of the palleal complex in a horizontal plane; while the second comprises those more recent theories of Pelseneer, Amaudrut, and Boutan, which regard asymmetry primarily as the concomitant of a twist which causes the palleal complex to move in a vertical plane.

Bütschli (4) was the first to put forward in an exact and careful way the point of view which is now common to all theories of the former class. He puts aside the older view of Spengel, which obviously runs counter to known embryo-

logical facts, by pointing out how the anus must have lain at all stages in the palleal groove, and then proceeds to build up his own theory of unequal growth, and the resulting gradual approximation of mouth and palleal complex on the right side. That such an approximation does take place in the ontogenetic history is, of course, well known, and the manner in which it is brought about seemed to Bütschli equally obvious. According to him, at a time when the anus lies in the middle line posteriorly, a narrow zone on the right side of the animal ceases to grow altogether, while the corresponding zone on the left grows with great vigour, and thereby the anus appears to be pushed up the right side of the body, while in reality the distance between it and the mouth remains always the same. Meanwhile the foot on the one hand, and the mantle on the other, continue to grow symmetrically. This process cannot by itself, however, bring about the crossing of the visceral connectives. For this Bütschli has to invoke the aid of the mantle cavity, which, he says, is formed rapidly at a time when the anus lies far forward on the right side of the body, and, by its growth backwards and to the left, carries the organs of the original right side of the body back with it and over the mid-dorsal line. All this he puts forward as ontogenetic fact, and therefore probable phylogenetic theory.

Bütschli's views have been adopted with more or less slight modification by many authors, and have recently been brought forward again with some additions by Plate (15). The great difficulty, to which no one could find a fully satisfactory solution, was the absence of any known cause of asymmetrical growth in a perfectly symmetrical body. Plate seeks an explanation in the asymmetry of the liver. Starting from the nearly symmetrical liver of the Chitons, and comparing it with the asymmetrical organ in the Gastropoda, he describes how, in the primitive form, a rapid growth of the left liver must have taken place at the expense of the right, which would result in the formation of a hernia posteriorly on the left side. Thus the first rudiment of a coil is formed,

which, for reasons connected with the equilibrium of the body, lies with its apex pointing towards the right (see his figs. F to H, pp. 185 and 187). This it is, he believes, which causes the approximation of the mouth and anus on the right side. As coiling proceeds this process would be accentuated, and so, apparently, the condition which obtains in the adult Gasteropod is reached without the aid of the late development of the mantle cavity relied upon by Bütschli. Though this is put forward merely as a phylogenetic theory, Plate believes that the facts of development will fully bear it out, and it is only from this point of view that we can deal with it here.

Pelseener (14) was the first to put aside the old point of view. To him it seemed that embryologically two distinct processes took place, both of which had for their object, as it were, the approximation of mouth and anus. The first of these, which he calls "torsion ventrale," leaves the embryo still symmetrical, but with the alimentary canal bent sharply so that the anus lies far forward ventrally. The mouth and anus being prevented from approaching nearer along this line on account of the outgrowth of the foot, the second process comes into play. This is a "torsion verticale," which takes place at right angles to the last, and has the result of all the organs contained in the shell undergoing a rotation through 180° , the ventral anus thereby becoming dorsal, the organs of the original right side being carried over to the left, and those of the original left to the right.

More recently Amaudrut (1) has approached the same problems from the point of view of comparative anatomy, and, from a study of the œsophagus and adjacent organs of a number of Gasteropods, has come to the conclusion that the region between the head and the visceral hump has undergone a twist through 180° . This, of course, would fit in well with Pelseener's observations, for, if the whole visceral hump has undergone torsion with regard to the head, the œsophagus must needs be twisted.

Finally, Boutan (2) has brought out a paper on the

asymmetry of the Gasteropods, which upholds essentially the same view of torsion for the Prosobranchs as Pelseneer and Amaudrut had already enunciated, and which derives its chief value from the author's claim to have actually observed the vertical torsion take place in the case of *Acmea*.

It is at once evident that the processes which, broadly speaking, characterise respectively the two classes into which we divided the theories under discussion, will not have entirely similar results. Both, indeed, alike have, as their chief results, the forward dorsal position of the anus and the crossing of the visceral connectives, for it was to account for these facts that the theories were originally framed; but, on the other hand, the twisting of the œsophagus, if true, could never have arisen from the processes which Bütschli describes, while the growth of the mantle must be conceived quite differently, according to which hypothesis is accepted. If Bütschli is correct, what was originally right remains on the right side throughout; while, according to the view of vertical torsion, the mantle, and therefore also the shell, share in the displacement of the palleal organs. The same holds good for most of the viscera, and is especially clearly illustrated in those organs which lie dorsally or ventrally; for while on the theory of unequal growth a lateral shifting might easily take place in the same manner as is the case for the palleal organs, a dorso-ventral displacement is only readily understood on such a theory as Pelseneer's. Thus Plate has to account for the gonad, which is dorsal in the Chitons, having a ventral position in the Gasteropoda, by supposing that a lobe of the great liver of the left side grew dorsally to it and pressed it against the foot.

Taking these considerations separately, and beginning with the last, the facts of embryology seem to me to show in a quite unequivocal manner that actual rotation of the organs has taken place round an axis coinciding with the œsophagus in its direction. It is for this purpose that the drawings of figs. 11 to 17 were made, and a comparison of these with one another, bearing in mind that they are all orientated on the

page in the same way with regard to head and foot, shows clearly how stomach, liver, pericardium, kidneys, and mantle cavity have all rotated in a perfectly definite manner, while retaining unaltered their relations inter se, and explains both the original dorsal and later ventral position of the gonad, without the intervention of any dorsally growing liver lobe. It is, moreover, striking that the torsion of the visceral connectives and the apparent twist of the œsophagus noticed on p. 119, keep pace perfectly with this rotation, so that it is almost impossible not to connect the two phenomena.

A comparison of figs. 16 and 17, on the other hand, causes some little difficulty, for although torsion seems to have taken place through an angle of very nearly 180° in fig. 16, there seems to be an apparent twist of about 90° further in fig. 17, and so it may seem that we have proved too much by this comparison of transverse sections. Two facts are, however, noticeable. In the first place, whereas up to Stage G, a transverse section of which is shown in fig. 16, the correspondence is perfect between the degree of torsion shown by the œsophagus and connectives on the one hand, and the rotation of the organs on the other, this is no longer the case in Stage H, where the connectives are only twisted through 180° , while the organs in the posterior region of the visceral hump have apparently rotated through about 270° . It is indeed true that an accessory twist has been noticed for the connectives as well as for the other organs in Stage H, but this is clearly marked off from the regular twist corresponding to that of earlier stages, which takes place more anteriorly; and a study of the development of the mantle cavity, and its disposition in this stage, makes it clear that the two twists are quite unconnected. The visceral commissure is formed from the floor of the mantle cavity, and, as the original right portion of the latter is carried far down on the left side of the body, so also is the origin of the corresponding half of the visceral commissure; while, as the original left side of the mantle cavity remains feebly developed, it, and consequently also the original left portion of the commissure,

lie more dorsally. The commissure thus comes to lie obliquely quite independently of any direct connection with the torsion of the body, and this irregularity is accentuated by a tendency on the part of the commissure to pass even more over to the left side of the body, and so place itself symmetrically with regard to the asymmetrical mantle cavity; or, in other words, symmetrically between its two extreme points of origin. This accessory twist may, therefore, be left out of account for the present purpose, and we are justified in saying that, as far as true torsion is concerned, the connectives only show a twist of 180° .

The second point to be noticed is that, whereas growth in the circumference of the visceral hump has been hitherto so slow as to be almost inconsiderable—for instance, between Stages D and G—though at the same time torsion has advanced rapidly, an enormous growth has taken place between Stages G and H. I believe that it is in these two facts that we find the solution of the difficulty. Between Stages G and H a kind of accessory or false torsion has taken place among the organs in the posterior region of the visceral hump, due merely to unequal growth amongst themselves, and not having as its concomitant further true torsion in the anterior region. If we seek further for the cause, I think we find it in the lengthening of the alimentary canal, and the great development of the stomach, which have together brought about the present relation between the stomach and liver, and also in the sudden rapid widening of the original left portion of the pericardium, which has hitherto been so narrow, a process which would have, as a natural result, the pushing of both kidneys more towards the definitive left side of the body than they were before. We have some evidence, then, for believing that the changes which are noticeable in the position of the organs in the body in successive stages are at first due to a rotation of the whole visceral hump upon the head through an angle of 180° , but that after this is complete a further apparent rotation affecting the posterior region of the body only is induced by unequal growth and

consequent rearrangement of the organs within the visceral hump. These two processes are, of course, quite distinct.

It has already been necessary to touch upon the subject of the torsion of the œsophagus. Amaudrut, as noticed above, has worked out with great care the twisting of the anterior aorta, the salivary glands, etc., about the œsophagus, and has come to the conclusion that this is due to an actual torsion of the region of the body between the head and visceral hump, but hitherto, so far as I know, no embryological evidence has come to hand. I have already described (p. 119) the curious compression of the œsophagus, and the manner in which, in transverse sections, the long axis changes its direction in *Paludina* embryos. This is not direct proof, but it is difficult to find any other explanation of the occurrence, except that it is due to the œsophagus being forced to undergo an actual twist. In quite old embryos, and in the adult, the œsophagus is no longer compressed, and this appearance is quite lost.

Now, granted that such a twist does take place, it follows, as Pelseneer points out, that originally i. e., in the untwisted forms, the shell, if coiled, must have been coiled exogastrically. It is exceedingly difficult to get any direct evidence upon this point, for, as Plate remarks, we cannot rely on the shell of any of the primitive Prosobranchs, like *Fissurella*, as these have all undergone torsion, and, on either view, an exogastric shell, if present, must be secondary; while, on the other hand, coiling does not begin to take place sufficiently early in the course of développement to give us clear evidence either way. It is, however, worthy of remark that all the monstrosities that I came across which, for some reason or other, had remained untwisted, if they showed any tendency towards coiling of the visceral hump at all, had begun to coil exogastrically. Of these the most highly developed is shown in fig. M II, and has been already described. There were, however, two others that showed a distinct tendency in the same direction, while I did not find one with anything like an endogastric coil. This fact seems to me highly significant.

The last of these broadly marked points of difference

between the two general theories under consideration relates to the symmetrical growth of the mantle, and this is the only one the evidence upon which seems still to point in favour of Bütschli's view; for, if the whole visceral hump has undergone a rotation, we should expect to find signs in the innervation. But the definitive right side belongs to the original left, and vice versa; whereas it is well known that the right pleural ganglion gives rise to the mantle nerve of the definitive right side of the body, and the left pleural ganglion to that of the definitive left. How this can be explained upon Pelseneer's view is not quite clear, unless it may be that the mantle nerve is embryologically a late outgrowth of the pleural ganglion, and is altogether post-torsional; but if this be so we shall have to admit a discrepancy between embryology and phylogeny. The case, however, is not altogether easy, even for the upholders of Bütschli's theory of the symmetrical growth of this region of the body, for the innervation is not wholly symmetrical. As Bouvier (3) remarks, "*la branchie n'est rien autre chose qu'une formation palléale, et les mêmes nerfs qui l'innervent se répandent en même temps dans le manteau,*" and, as is well known, the definitive left ctenidium is innervated from a ganglion belonging to the original right side of the body. The question, then, is one of some difficulty whichever view one takes, and of hardly greater difficulty in the one case than in the other.

An examination of the broad features of the two great classes into which we divided the theories of Gasteropod torsion leaves, then, a balance of embryological evidence in favour of that class of which Pelseneer was the first exponent. It will be well now to examine the individual theories more in detail. Bütschli's views have already undergone criticism at the hands of Pelseneer and others as not corresponding to the facts of ontogeny as one sees them. Amaudrut criticises him more particularly with regard to the position of the supra- and sub-intestinal ganglia. The supra-intestinal ganglion, he says, is usually situated further back than its

fellow on the other side of the body. Now, as the former was originally on the right side of the body, it would fall into the zone where Bütschli supposes that growth ceases, while the subintestinal ganglion, on the other hand, would be originally in a zone of very active growth. This, Amaudrut argues, should lead to the subintestinal ganglion being pushed the further back of the two, while precisely the opposite is actually the case. If, however, we suppose that instead of the left it was at first the right side which grew most actively the existing condition of affairs would be obtained; and as, after torsion, the zone of active growth would be transferred from the right to the left, we probably find an explanation of Bütschli's error in his having examined too late stages of development. The argument concerning the position of the ganglia seems sound so far as it goes, but the alleged reason of Bütschli's mistake is not so easy to accept, seeing that he starts from a form in which the anus lies in the middle line behind, and in which even the ventral flexure has apparently not yet begun.

Bütschli himself relies for his evidence of unequal growth upon having demonstrated that in different stages of development the mouth and anus do, as a fact, remain exactly the same distance apart while the body is increasing in size and torsion is taking place. Now, it seems to me that the value of this argument depends largely upon the view we take of the development of the mantle cavity. If we regard it as von Erlanger did, and as I believe we must regard it, as the result of an outgrowth of the mantle rather than as an invagination of the surface of the body, Bütschli's argument is entirely destroyed, for then it is not the mouth and anus that we must compare so much as the mouth and the back of the mantle cavity; and the fact that the anus lies near the outer edge of the mantle cavity shows only that rapid growth of the rectum has been taking place in this region, and has caused a closer approximation of the anus to the mouth than would otherwise have been the case. Whichever view we

may take of this matter, however, we are forced to admit that rapid development of the mantle cavity must mean rapid growth in the neighbourhood of its formation ; and we have already seen in the descriptive part how a rapid formation of the mantle cavity takes place between Stages D and E to the original right of the anus, that is, in Bütschli's zone of cessation of growth, and this long before torsion is complete. His view of the late formation of the mantle cavity and its effect upon the position of the organs seems to me no less in entire contradiction to embryological fact. The mantle cavity is not formed by any means so late as Bütschli would put it ; it takes its origin, in fact, almost simultaneously with the first appearance of torsion, and is, as we have seen, strongly developed before we reach Stage F. Finally, it is, of course, impossible that its formation should have the effect attributed to it by Bütschli unless we regard it altogether as an invagination, which we have not sufficient evidence for doing.

All the above remarks apply, also, to Plate's theory, since he accepted Bütschli's in its main features. The former's suggestion, however, that the liver is the first cause of asymmetrical growth is an interesting one, and must be further examined. His working out of the later stages of development, the formation of the hernia, and the production of the coil find no support in embryology. A hernia, indeed, is formed on the left side in the development of *Paludina*, but this is of quite a different nature from that described by Plate, as a comparison between his figures and mine will show ; for whereas in his theoretical form the hernia contains only the liver of one side, and is at the same time the beginning of the coil of the visceral hump, the bulging out of the side of the body in *Paludina* is, as the figures show, equivalent to the once symmetrical apex of the visceral hump, and contains the stomach as well as the liver. It is, moreover, in no way comparable to the formation of a true coil, which is formed by a distinct outgrowth of the liver at a later stage (cf. figs. 13 and 16 with fig. 17). The coil in *Paludina* does not begin till torsion is nearly complete, and

therefore it cannot form an ontogenetic cause for the forward movement of the pallear complex. Phylogenetically the evidence is only negative, but at least embryology gives no support to this part of Plate's theory.

Putting aside, however, the question of the coiling of the visceral hump, if we follow the progress of torsion from stage to stage we can, I think, in no way regard this as dependent upon the growth of the liver in each stage. Comparing Stages C and D, for instance, both as complete embryos and in sections (cf. especially figs. 11 and 12), we find the slight growth of the liver more than counterbalanced by the great development of the pericardium and mantle cavity, so that whereas in fig. 11 a line joining the junction of the stomach and liver with the ridge *x*, which, for our present purposes, may be taken to represent the position of the rectum, divides the section into very nearly equal portions, a similar line in fig. 12 makes that portion which contains the liver considerably smaller than the other, which contains the great original right extensions of the pericardium and mantle cavity. In the next stage (fig. 13) this has been partly rectified by rapid growth of the liver, but in Stage F the inequality is again very marked, and, in fact, from Stages E to G the growth of the liver is very slight, while torsion is rapid.

While entirely repudiating the idea, however, of the liver acting, as it were, as the propelling force throughout ontogeny, it may yet be possible to agree with Plate in regarding it as the original disturber of symmetry; and some support is lent to this view not only by the very early asymmetry of this organ, but also by the fact that it is not present, or is only very slightly developed, in the symmetrical monstrosities. But it should, at the same time, be noticed that the development of *Paludina* gives no more support to this part being acted by the liver than by the mantle cavity. The latter organ also is asymmetrical from the time of its first formation in normal forms; while in Monstrosity III, which was slightly twisted towards the left, the mantle cavity is considerably developed in a manner to correspond to the torsion, while the

liver is comparatively insignificant. Moreover, in following the normal development from stage to stage, it is obvious that the great original right-hand extension of the mantle cavity much more nearly keeps pace with the torsion than is the case with the liver; and, finally, it may be noticed that this view would harmonise with Amaudrut's reasoning concerning the supra- and sub-intestinal ganglia. It may well be, however, that the growth of these organs is to a large extent dependent the one upon the other, and that equilibrium is maintained by the asymmetrical growth of the liver on one side of the body being compensated by asymmetrical growth of the mantle cavity on the other.

We turn now to the other side of the question, but Pelseneer puts forward his theory in such a broad and general form that it is difficult to enter into any detailed discussion beyond the general considerations which have already been adduced in his favour. The chief objection that might be raised in this case is, perhaps, one which arises from the difficulty, in a course of development like that of *Paludina*, of distinguishing Pelseneer's two processes of ventral and lateral torsion. And, indeed, they do go on so closely hand in hand that as a matter of fact the anus travels in an oblique and never in a vertical direction. Nevertheless there is clear evidence of a vertical rotation of the organs contained in the visceral hump, as has been already pointed out, and in each stage it is possible to separate from the results of this process a certain clearer development of the visceral hump and sharper bend of the alimentary canal from that which obtained in the previous stage, which must be the result of a process akin to Pelseneer's ventral torsion, or, as Amaudrut has better styled it, ventral flexion. In the early stages the distinction between the two processes is very clear—as, for instance, in Stage C, where the ventral flexion is already strongly marked, while the lateral torsion is but just begun.

With regard to the cause which Pelseneer seeks for these processes, however, the development of *Paludina* offers no confirmation. The growth of the foot, he says, forms an

obstacle to the close approach of the mouth and anus, and therefore vertical torsion takes the place of the ventral flexure. This is really no true explanation at all, for we are left in the dark as to how the foot brings about this new state of affairs, and we do not get much nearer if we say, with Boutan, that there is an antagonism of growth between the foot and the visceral hump. It seems to me that in the development of *Paludina* it is altogether out of place to speak of such an antagonism, for torsion begins at a time when the foot is still but a comparatively insignificant ventral projection, and long before the formation of the creeping sole.

Amaudrut also is unsatisfactory in this respect, for he attributes torsion ultimately to voluntary effort on the part of the animal to get its gills into a better situation. With the main part of Amaudrut's paper embryology has nothing to do; there are, however, one or two points in which it seems to me he is mistaken, owing to a too exclusive regard for the anterior region of the body. His description of the shell and visceral hump as aiding the torsion by their weight is wholly inapplicable to embryology; while his account of the manner in which the peculiar shape of the mantle cavity and the disposition of the organs included in it are induced receives complete contradiction. "*À peu près dans le même plan transversal qui passe pas le ganglion viscéral postérieur,*" he says, "*se trouvent la partie terminal de la région tordue du tube digestif, le fond de la cavité respiratoire, la partie postérieure de la branchie et le cœur. Ce plan marquant en arrière la limite extrême de la torsion, les organes qui s'y trouvent ont dû exécutés un mouvement de rotation d'environ 180° pour se rendre dans leur position définitive.*" This holds good for all organs behind this position, but in front of it obviously torsion will be less. It is these facts of which he makes use to explain the apparent slope of the mantle cavity from the left towards the right anteriorly, and of the rectum from mid-dorsal, where theoretically it should be, to the right side of the body, the characteristic position for the Prosobranchia. In Stage II, however, the region of the

twist of the œsophagus and visceral connectives lies altogether in the anterior region of the mantle cavity, and in front of the anus. All the region behind this, we must believe, has equally undergone a torsion of 180° , and yet here we have clearly marked that peculiar disposition of the mantle cavity which Amaudrut seeks to explain. It seems to me that this is not to be regarded as due to torsion at all, but to unequal growth; already in Stage E, as we have seen, the main features were present, the mantle cavity reaching over the mid-dorsal line behind and being much less advanced in front, while the rectum showed no tendency to a corresponding disposition. It is not, in fact, till Stage H that the position of the anus ceases to be where theoretically it should be according to the degree of torsion, and here the displacement is due to a sudden bend of the rectum to the right quite close to the anus. The final disposition of the rectum is due to a forward growth in the direction indicated by this bend after torsion is complete, and may perhaps be due, phylogenetically, to the advantage gained by the animal in having the anus in a position as far removed from the gill as possible.

Boutan's explanation of the cause of torsion has already been mentioned, and it has been shown to be hardly applicable in the case of *Paludina*. His conception of this antagonism of growth between visceral hump and foot is, much more than Pelseneer's, that of an ontogenetic cause, for he expresses the opinion most definitely that if this antagonism could be suppressed torsion would not take place. Now amongst the monstrosities already referred to it is certainly true that I did not find one with an abnormally small foot and yet a visceral hump which had undergone torsion, but, on the other hand, fig. M 11 shows how both foot and hump may be very highly developed and yet no torsion take place. Surely the antagonism of growth, if such exist, must be much greater in this case than, for instance, in Stage C, where torsion has already begun. This leads on to Boutan's view of torsion as the cause of asymmetry.

The normal Gasteropod larva, he says, is perfectly bilaterally symmetrical, and remains so till torsion takes place; but as soon as this begins the asymmetrical growth of the internal organs begins, and, if torsion could be averted, symmetry would be maintained. Certainly in this connection the symmetry of Monstrosity II is striking, but that asymmetry and torsion are closely connected no one doubts, and whichever were the cause of the other, or if both were the outcome of some common cause, the result would be the same. In the normal course of development it has been repeatedly pointed out by other writers that asymmetry in some form or other is found before torsion begins. In *Paludina* torsion begins so early that it is difficult to be quite sure of this, but the liver, at least, is never wholly symmetrical, and the unequal development of the original rudiments of the mantle cavity takes place as nearly as possible simultaneously with the beginning of torsion.

Once more, Boutan turns to antagonism between foot and visceral hump in order to explain the coiling of the latter. If, when the creeping sole of the foot is developed, this can stretch out without lateral displacement of the visceral hump, then the shell, he believes, will remain symmetrical; but if not, then the hump will be pushed to one side or other, and the sense of the future coil will depend upon which side it is pushed towards. Such a view, it would seem, would be quite impossible to accept after even a cursory view of the facts of development. For if, as would seem to be the case, the coiling of the visceral hump is primarily the result of a definite process of growth in the liver (cf. figs. 16 and 17), this is altogether independent of the exact relation in which this organ finds itself to the foot. As a matter of fact, the dextral coil of *Paludina* begins before torsion is quite complete, and therefore, while the apex of the visceral hump is still to the left of the foot, by a strong growth of the liver towards the right, which, when torsion is complete and the visceral hump nearly symmetrical, points to the right and upwards. It is altogether inconceivable that any accident of

growth, independent of torsion, which should cause the apex of the visceral hump to remain upon the left side of the foot, should alter this growth of the liver, and cause the coil to become sinistral.

With regard to the cause of asymmetry, another view remains to be mentioned here, namely, that of Grobben (8), who, while accepting Pelseneer's main conclusions, finds himself unable to regard the antagonism between foot and visceral hump as a true ontogenetic cause of torsion. He grants Pelseneer's view that the growth of the foot necessitates a vertical displacement if the anus is to continue to approach the mouth, and in order to explain how this is produced he has recourse to Plate's suggestion of an unequal growth of the two originally symmetrical liver lobes. For reasons already stated when Plate's theory was under discussion this is not altogether satisfactory, for the growth of the liver does not keep pace with torsion, and the chief development of it takes place after torsion is complete. As a phylogenetic cause it may have played its part, but probably not quite in the manner that is described by Plate.

Thiele (18) also takes up a position somewhat intermediate between the two extreme points of view on torsion, for, while agreeing with Pelseneer that before torsion the shell must have been bent with its apex pointing forwards, as though forming the beginning of an exogastric coil, he approaches Lang more nearly than anyone else in his view of how torsion has been effected. He dismisses Plate's view of the liver as the disturber of the original symmetry of the body, and believes this part to be played by the gonad, in which he also sees the cause of the coiling of the visceral hump. Thus, as, in the Gasteropods, the gonad is formed only on the left, the coil also lies with its apex in the left. The condition of affairs which is thus reached is shown in his fig. 3, p. 15, and this he believes to be a position of unstable equilibrium, consequently a sudden rotation is effected till the visceral hump comes to rest in the normal adult position.

Thiele seeks very little support for his view in the facts of development, except in the rapid rotation observed by Boutan in *Acmea*, and it is seen at a glance to be wholly inapplicable to the development of *Paludina* where rotation is gradual, and where both gonad and coil are only formed when torsion is far advanced. It brings us, therefore, no nearer to forming a conception of the ontogenetic course of torsion, and the development of *Paludina* gives no evidence to support it as a phylogenetic theory.

Whatever view may be held with regard to phylogeny, in ontogeny it seems to me that we are ultimately thrown back upon the problems of heredity, and for the present we must agree with Guiart (9) when he says, "Mais à ceux qui nous demanderont la cause mécanique de cette torsion, et qui nous reprocheront de ne pas l'avoir trouvée chez l'embryon, nous répondrons simplement ceci. Il ne faut pas confondre ontogénie et phylogénie, la cause n'existe pas chez l'embryon, mais chez le mollusque primitif." From the nature of the case the evidence which ontogeny can give upon the phylogenetic cause is merely negative.

SUMMARY.

To sum up, then, theories of Gasteropod torsion may be divided into two classes:

a. Those which view the present position of the pallial complex as due to a forward movement along the right side of the body, which resulted from greater growth of the left side of the body than of the right.

b. Those which view the present position of the pallial complex as due to a ventral flexion followed by a vertical rotation of the whole visceral hump upon the head.

The evidence for the second of these views seems greater than that for the first, in that—

1. A vertical displacement through 180° of all the organs contained in the visceral hump takes place in the course of ontogeny.

2. There is some evidence, both from comparative anatomy and embryology, for believing that the œsophagus has undergone an actual twist.

3. Monstrosities which retain the palleal complex in a ventral position show a tendency to form an exogastric coil.

The innervation of the mantle was shown to be equally difficult to explain on either hypothesis.

Also, against the first view was urged the insufficiency of the evidence upon which Bütschli bases his conclusions with regard to zones of unequal growth.

With regard to the phylogenetic cause of the vertical twist, embryology can only give negative evidence; while in considering the ontogenetic cause we are thrown back upon unsolved problems of heredity, and must confess our ignorance.

In conclusion, I wish to offer most hearty thanks to Professor Weldon, not only for having placed freely at my disposal all the resources of the laboratory, but also for most kind personal aid at all stages of the work. My thanks are also due to Mr. Richard Evans for much help in the technique.

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DESCRIPTION OF PLATES 7—9,

Illustrating Isabella M. Drummond’s paper, “Notes on the Development of *Paludina vivipara*, with special reference to the Urinogenital Organs and Theories of Gasteropod Torsion.”

Significance of Reference Letters.

a. Anus. *aa.* Line representing a median ventral plane through head and foot. *ect.* Outer epithelium of the body. *f.* Foot. *g.* Gonad. *h.* Heart. *k.* Kidney. *r. k.* Original right kidney. *l. k.* Original left kidney. *k. d.* Kidney duct. *l.* Liver. *m.* Mouth. *m. c.* Mantle cavity: *r. m. c.* Original right horn; *l. m. c.* Original left horn. *m. f.* Mantle fold. *æs.* Œsophagus.

op. Operculum. *ot.* Otoecyst. *pc.* Pericardium: *r. p. c.* Original right division; *l. p. c.* Original left division. *p. g.* Pedal ganglion. *p. n.* Pedal nerves. *r. ap.* Renal opening into the mantle cavity. *rec.* Rectum. *r. g. ap.* Reno-gonadal aperture. *r. pc. ap.* Reno-pericardial aperture of the original left kidney. *r. s.* Radula sac. *s. g.* Shell gland. *st.* Stomach. *sub. c.* Sub-œsophageal connective. *sup. c.* Supra-œsophageal connective. *t.* Tentacle. *v.* Velum. *ves.* and *ves'.* Vesicles attached to kidneys. *v. h.* Visceral hump. *x.* Ridge between the two original horns of the mantle cavity on which the anus opens.

PLATE 7.

FIG. 1.—Slightly oblique transverse section through the visceral hump of an embryo between the age of that shown in Fig. C and that in Fig. D. Both kidneys and the first rudiment of the heart are shown. $\times 330$.

FIG. 2.—Oblique section through the extreme (original) left portion of the pericardium of an embryo rather older than that shown in Fig. G. The sharp curve of the surface of the body shown at *ect.* indicates that the visceral hump is just beginning to coil. $\times 330$.

FIG. 3.—Another section of the same series, and showing the same region, but rather more posterior. Two sections intervene between Figs. 2 and 3. $\times 330$.

FIG. 4.—The next section posterior to Fig. 3, and showing the same region. $\times 330$. The figure should be rotated about 9 degrees to the left to compare with Fig. 3.

FIG. 5.—A transverse section through the same region as the above, but of an older embryo in which about one complete turn of the spiral coil of the visceral hump is complete. It is one of the same series as Fig. 17. $\times 330$.

FIG. 6.—An ideal longitudinal section of the whole genital apparatus of an advanced embryo, with about two turns of the spiral complete, reconstructed from a series of transverse sections. The gonad is represented spread out instead of coiled. $\times 140$.

FIG. 7.—A transverse section across the region *aa.* of Fig. 6. $\times 330$.

FIG. 8.—A transverse section across the region *bb.* of Fig. 6, showing also the close proximity of the genital organs to the liver. $\times 330$.

FIG. 9.—A transverse section across the region *cc.* of Fig. 6. $\times 330$.

FIG. 10.—Sagittal section through an embryo belonging to Stage A. $\times 330$.

FIG. 11.—Transverse section through the posterior region of the visceral hump of an embryo belonging to Stage C. $\times 119$.

FIG. 12.—Transverse section through the posterior region of the visceral hump of an embryo belonging to Stage D. $\times 119$.

FIG. 13.—Transverse section through the posterior region of the visceral hump of an embryo belonging to Stage E. $\times 119$.

FIG. 14.—Another section from the same series but passing through the anterior region of the visceral hump, and showing the great anterior or right extension of the mantle cavity. $\times 119$. The section of the overhanging foot, *f*, is introduced here in its relative position, but is omitted in Fig. 13.

FIG. 15.—Transverse section through the posterior region of the visceral hump of an embryo belonging to Stage F. $\times 119$.

FIG. 16.—Transverse section through the posterior region of the visceral hump and foot of an embryo belonging to Stage G. $\times 119$.

FIG. 17.—Transverse section through the posterior region of the visceral hump and foot of an embryo belonging to Stage H. $\times 119$.

FIGS. 18 and 19.—Transverse section through the "neck" region of the same stage, showing the twist of the visceral connectives. $\times 119$.

FIG. 20.—Transverse section through the visceral hump of the monstrosity shown in Fig. 10, Plate 8, in the region of the kidneys. $\times 87$.

FIG. 21.—Another section of the same series passing through the opening of the kidney duct into the mantle cavity. $\times 87$.

FIG. 22.—Transverse section through the stomach of the same embryo just behind the opening into it of the œsophagus. $\times 80$.

FIG. B.—View of an embryo belonging to Stage B from the right side. $\times 70$.

FIG. C.—View of the right side of an embryo belonging to Stage C. $\times 70$.

FIG. C₁.—View of the ventral surface of an embryo slightly older than the last. $\times 70$.

FIG. D.—View of the left side of an embryo belonging to Stage D. $\times 70$.

FIG. E.—View of the left side of an embryo belonging to Stage E. $\times 70$.

FIG. E₁.—View of the right side of the same. $\times 70$.

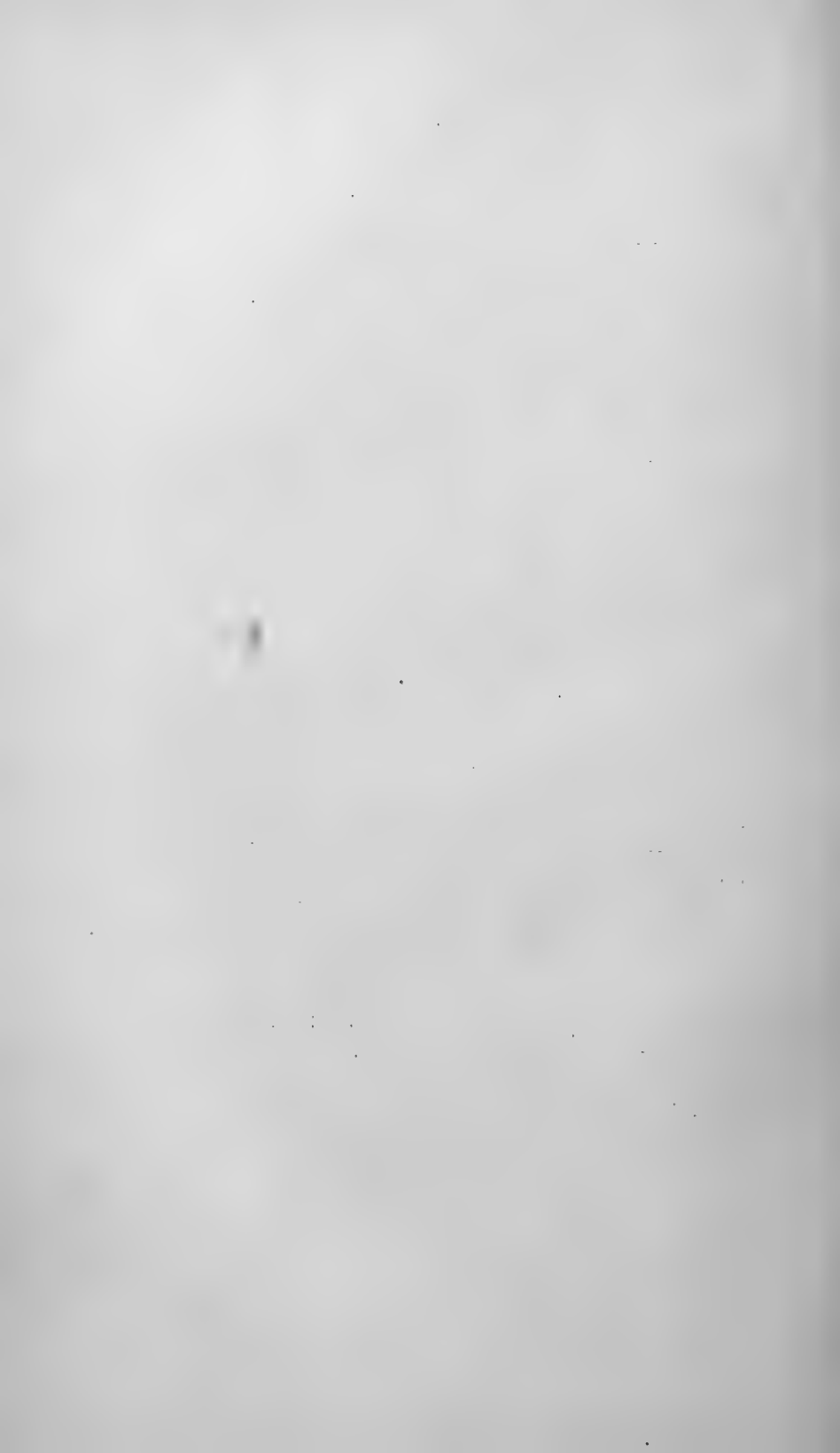
FIG. E₂.—Dorsal view of the same. $\times 70$.

FIG. F.—View of the left side of an embryo belonging to Stage F. $\times 70$.

FIG. G.—View of the left side of an embryo belonging to Stage G. $\times 70$.

FIG. M II.—View of the left side of Monstrosity II. $\times 70$.

FIG. M III.—View of the left side of Monstrosity III. $\times 70$.



Is Chemotaxis a Factor in the Fertilisation of the Eggs of Animals?

By

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I. INTRODUCTION.

THE well-known researches of Pfeffer¹ have demonstrated the importance of the part played by chemotactic stimuli in causing the spermatozoa of liverworts, mosses, ferns, etc., to approach the oospheres. Among the yet higher plants—Gymnosperms and Angiosperms—the chemotropism² of

¹ "Locomotorische Richtungsbewegungen durch chemische Reize," 'Untersuchungen aus d. Bot. Inst. zu Tübingen,' 1884, Bd. i, p. 363.

² Molisch, "Ueber die Ursachen der Wachstumsrichtungen bei Pollenschläuchen," 'Sitzungsber. der Kais. Acad. d. Wiss. in Wien,' 1889 and 1893. Also Lidforss, "Ueber den Chemotropismus der Pollenschläuche," 'Ber. d. D. Bot. Gesell.,' 1895, Bd. xvii, p. 236.

pollen-tubes takes the place of the chemotaxis of spermatozoa. We may therefore say that actual contact of the sexual elements of all plants from the liverworts onwards is brought about by chemical stimuli.

In all the above-mentioned groups of plants the oospheres are fertilised in their place of origin without being set free. The chemical stimulus, so far as is known, does not arise directly from the oospheres. The spermatozoa of the vascular cryptogams are attracted into the archegonia by a substance liberated from the cell-sap of the neck-canal-cells. The pollen-tubes are guided on their tortuous way to the oospheres by substances excreted by various tissues of the ovary and ovules.

On the other hand, the ova of animals are fertilised after being set free from their place of origin, namely, the ovary. Fertilisation takes place in the case of terrestrial animals, e. g. mammals, reptiles, birds, and insects, in the oviduct, or, as happens with many aquatic animals, e. g. Echinoderms, many fishes, and amphibia, after the eggs have been deposited in water. If, therefore, chemotaxis plays a rôle in bringing the spermatozoa of animals into contact with the ova, the source of stimulation must be looked for in a substance excreted from the eggs.

It appears to be the general opinion among zoologists that chemotaxis is actually a factor in the fertilisation of animal eggs. Thus Bergh¹ says that during an artificial fertilisation experiment, e. g. in the case of the sea-urchin, "the spermatozoa collect around the ripe eggs, probably attracted by a special substance."

Wilson,² in his latest edition of 'The Cell,' in dealing with the union of the germ-cells, remarks: "There is clear evidence of a definite attraction between the germ-cells, which is in some cases so marked (for example, in the polyp *Renilla*) that when spermatozoa and ova are mixed in a small

¹ Bergh, 'Vorlesungen über allgemeine Embryologie,' 1895, p. 43.

² Wilson, 'The Cell in Development and Inheritance,' 2nd ed., 1900, p. 196.

vessel, each ovum becomes in a few moments surrounded by a dense fringe of spermatozoa, attached to its periphery by their heads, and by their movements actually causing the ovum to move about. The nature of the attraction is not positively known, but Pfeffer's researches on the spermatozoa of plants leave little doubt that it is of a chemical nature. The experiments indicate that the specific attraction between the germ-cells of the same species is owing to the presence of specific chemical substances in each case." Here it may be at once remarked that the collection of spermatozoa attached by their heads to the eggs in artificial fertilisation experiments is no proof whatever that the spermatozoa have been attracted from a distance to the egg by a substance excreted from the latter. All that we can say in such a case, without further observation, is that the eggs retain the spermatozoa after these have come in contact with them.

Verworn¹ goes so far as to say: "The splendid and methodical researches of Pfeffer upon chemotropism had their origin in observations upon the spermatozoa of forms in which chemotropic relations to the egg-cell were discovered. Such relations, as we now know, have analogies in almost the whole of living nature and for the fertilisation of the eggs of animals by spermatozoa, just as for the eggs of plants, form an indispensable condition. The spermatozoon seeks the egg and is guided on the right course everywhere in the living world by a chemotropic action, which the metabolic products of the eggs exercise upon the free-swimming spermatozoa. That from the innumerable hosts of spermatozoa of the most diverse animals which in many places cloud the sea, each species finds its right and specific egg, a phenomenon which would otherwise excite astonishment, is in the great majority of cases a direct result of chemotropism, and easily explains itself on the ground that each spermatozoon is chemotropically attracted by the specific substances which characterise the eggs of the species concerned." It is one of the objects

¹ Verworn, 'Physiologie,' 1895, p. 425.

of this paper to show that such sweeping generalisations with regard to animals are so far entirely without experimental justification.

With mosses, ferns, etc., there is as yet no proof that the eggs attract the spermatozoa to them in the manner in which Wilson, Verworn, and others believe to be the case with animals. As was pointed out, the eggs of these plants are fertilised in their place of origin. This permits of the surrounding cells, neck-canal-cells, and ventral canal-cell taking upon themselves the function of chemically attracting¹ the male sexual element to the female. The eggs may not do more than simply retain the spermatozoa after contact has taken place. Since the eggs of animals are fertilised after liberation from their place of origin, there is no chance of such a division of labour as occurs with plants. In the analogy made by zoologists between ferns and animals there is thus a weak point. Credit is given to the reproductive egg of animals for an excretory function, which has not been demonstrated in the case of plants.

There is one group of Algæ—the Fucaceæ—which are unique among plants in that their eggs, like those of the Echinoidea, are fertilised after extrusion into water. The eggs of the Fucaceæ differ, however, from those of most animals, in being perfectly naked during fertilisation, and in containing chlorophyll which assimilates² in the light.

¹ The neck-canal-cells and ventral canal-cell secrete in the cell-sap of their vacuoles an attractive substance or substances (probably a salt or salts of malic acid). When the archegonium bursts these cells burst too, and die, thus liberating their cell-sap, which diffuses slowly out of, and from, the mouth of the archegonial tube. Pfeffer, loc. cit.

² This fact I was able to prove by means of Engelmann's method, using, however, the spermatozoa of a sea-urchin instead of bacteria. A vast number of spermatozoa were added to a preparation containing a few eggs of *Cystocira barbata* (one of the Fucaceæ). The spermatozoa not in the neighbourhood of the eggs came to rest in five minutes. Those around the eggs continued in motion for more than an hour. The movement also took place around non-nucleated fragments of eggs. When the light was cut off from the eggs the movement quickly ceased, to return again when light was once more admitted.

According to Strasburger¹ they excrete a substance which attracts the spermatozoa from a distance equal to two diameters of an egg. On the other hand, the observations and experiments by Bordet² upon the fertilisation of the eggs of several species of *Fucus* led him to entirely negative conclusions as regards a chemotactic attraction, while he found that the spermatozoa were highly sensitive to contact. According to this observer it is simply the ability of the spermatozoa to adhere to surfaces by the tip of one of their two cilia, which leads to their collection upon an egg, while their meeting with it is simply a matter of chance. A few observations of my own at Naples upon the fertilisation of *Cystocira barbata* (one of the *Fucaceæ*) did not reveal to me any certain attraction of the spermatozoa from a distance, but the collection of the spermatozoa upon the eggs in consequence of their ability to cling to surfaces was clearly seen. Nevertheless, in view of the positive statement of Strasburger, a careful reinvestigation of the question seems to me desirable.

The other cases³ of supposed attraction of spermatozoa to the egg-cells of plants all await a critical study.

In the cases of *Clamydomonas* and of *Ulothrix*,⁴ Pfeffer has observed that the meeting of the swarm-spores, which afterwards copulate, is purely a matter of chance. He also found that the spermatozoa of a bull⁵ were not attracted by meat extract.

At present, to the best of my knowledge, not a single case is known where chemotaxis plays a rôle in the fertilisation of the eggs of animals.

Dewitz⁶ has shown that the spermatozoa of certain insects

¹ Strasburger, 'Das bot. Practicum,' 2 Aufl., 1887, p. 402.

² Bordet, "Contribution à l'Étude de l'Irritabilité des Spermatozoïdes chez les Fucacées," 'Bull. de l'Acad. Belgique,' 3e sér., tome xxvii, 1894, p. 889.

³ See Pfeffer, loc. cit., pp. 446—449.

⁴ Loc. cit., p. 447.

⁵ Loc. cit., p. 449.

⁶ Dewitz, "Ueber Gesetzmässigkeit in der Ortsveränderung der Spermatozoen und in der Vereinigung derselben mit dem Ei," 'Arch. f. die gesammte Physiologie,' Bd. xxxviii, 1886, p. 358.

find their way to and through the micropyles of the eggs owing to the remarkable fact that on coming to a surface they remain in contact with it and continue to move in circles. This characteristic, which will be discussed more fully afterwards, I have found also shared by the spermatozoa of representatives of every group of the Echinodermata.

Massart¹ made a careful investigation of the fertilisation of frogs' eggs. He came to the conclusion that the spermatozoa come in contact with the gelatinous coat by accident, and cling to it owing to a special sensibility to contact. He found that they bore through it radially. He believed that this is explained on the supposition that the spermatozoa seek to bore from the more watery outer layers to the less watery inner layers in consequence of a sensibility to the differences of saturation.

My own investigations, undertaken at Naples, were made to determine the nature of the forces which bring the spermatozoa and eggs of the Echinoidea in contact, especial attention being paid to the chemotactic question. The work was taken up after a fairly extended study of the chemotaxis² of the spermatozoa of ferns.

II. SOME FERTILISATION PROBLEMS.

In the case of such eggs as those of the Echinoidea, which are surrounded by a thick gelatinous coat, some of the physiological questions that may be asked in regard to the manner in which the spermatozoa meet and fuse with them are as follows:

1. Does a spermatozoon meet the gelatinous coat (*zona pellucida*) by accident, or is it attracted to it by some

¹ Massart, (1) "Sur l'Irritabilité des Spermatozoïdes de la Grenouille," 'Bull. de l'Acad. roy. de Belgique,' 3me sér., t. xv, No. 5, 1888; (2) "Sur la Pénétration des Spermatozoïdes dans l'Œuf de la Grenouille," 'Bull. de l'Acad. roy. de Belgique,' 3me, sér., t. xviii, No. 8, 1889.

² Buller, "Contributions to our Knowledge of the Physiology of the Spermatozoa of Ferns," 'Ann. of Botany,' vol. xiv, 1900, p. 543.

chemotactic substance which is excreted by the living egg and diffuses through the gelatinous coat into the surrounding water?

2. After a spermatozoon has come in contact with the outer surface of the gelatinous coat, is it retained there mechanically or in consequence of a tactile stimulus exerted upon it by the surface?

3. Does the spermatozoon bore through the gelatinous coat radially? If so, why?

4. After reaching the outer surface of the living egg (i. e. the protoplasm), what is the nature of the forces which lead the spermatozoon to unite with it?

5. Closely connected with the latter is the further question: by what means is the progress of a spermatozoon from the surface to the interior of an egg brought about?

III. MATERIAL.

The following species of Echinodermata were made use of:

Class Echinoidea	{	Echinus microtuberculatus, Bly.
		Sphærechinus granularis, Ag.
		Arbacia pustulosa, Gray.
		Strongylocentrotus lividus, Brdt.
Irregulares	{	Echinocardium cordatus, Gray.
Class Asteroidea	{	Asterias glacialis, O.F.M.
		Echinaster sepositus, Müll. Tr.
Class Ophiuroidea	{	Ophioderma longicauda, Müll. Tr.
		Ophioglypha lacertosa, Lyman.
Class Holothuroidea	{	Holothuria Stellate, D.Ch.
Class Crinoidea	{	Antedon rosacea, Norman.

Observations upon the motility, especially in regard to surfaces, were made upon the spermatozoa of all the above species. The experiments and observations upon fertilisation were restricted to the first three Echinoidea, namely, Echinus, Sphærechinus, and Arbacia.

IV. REMARKS UPON THE EGGS AND SPERMATOOZOA OF THE ECHINOIDEA.

The eggs of the Echinoidea (as is also the case with all the Echinodermata) are surrounded by a thick, very transparent, gelatinous coat, the zona pellucida, through which the spermatozoa have to make their way before they reach the living protoplasm of the egg.

The following measurements from *Echinus* will give some idea of the relative sizes of the living eggs, the gelatinous

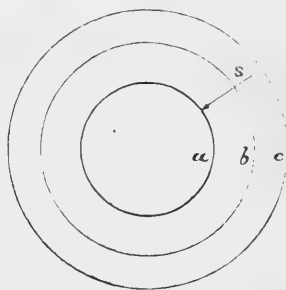


FIG. 1.—Egg of *Echinus microtuberculatus*. $\times 170$. *a*. Outline of protoplasm. *b*. Outline of zona pellucida after five minutes in sea-water. *c*. Outline of zona pellucida after several hours in sea-water. *s*. Spermatozoon.

coat, and the spermatozoa:—Diameter of living egg alone 0.11 mm.; diameter of living egg and gelatinous coat 0.18 mm.; thickness of gelatinous coat 0.036 mm.; length of a spermatozoon 0.051 mm.

Each of the measurements just given is the average of ten measurements. The eggs were measured almost directly after being placed in water.

The jelly increases in thickness after deposition of the egg in sea-water. After twenty-four hours it is found to have nearly doubled in thickness, and to have become 0.057 mm. wide. The width of the jelly, which in the fresh

egg is less, is, then, in the eggs which have stood twenty-four hours in water, slightly more than the length of the spermatozoa (Fig. 1).

The presence of the gelatinous coat is quite unessential to the union of spermatozoa and eggs, for if by shaking it be removed, fertilisation will still take place with the greatest ease.

When liberated at the top, the eggs gradually sink to the bottom of a beaker containing still sea-water. They thus appear to be heavier than their normal medium. Very small currents are, however, sufficient to keep the eggs floating. Probably in the sea, where the eggs are liberated, such currents are always present. In that case, too, the currents are of considerable importance in mixing the eggs and spermatozoa.

V. THE CHEMOTACTIC QUESTION.

After repeated trials with unilateral illumination, I was unable to detect the least sensitiveness of the spermatozoa of *Arbacia* or *Echinus* for heliotactic stimuli. It was therefore not possible by application of such a stimulus to allow the spermatozoa to stream in the direction of the eggs (which may be done in the case of *Fucus*), and to observe whether, when passing, they deviate toward them.

A large number of artificial fertilisation experiments were undertaken. To one side of a drop, either open or under a raised coverglass, a small drop bearing spermatozoa was added. The spermatozoa spread quickly in all directions, and in the course of their wanderings came in contact with the eggs, bringing about fertilisation. Within a few minutes this was made evident by the raising of the vitelline membrane. I failed, however, to observe any attraction of the spermatozoa toward the eggs from a distance, or any collection of the spermatozoa around the eggs outside the gelatinous coat. On the other hand, spermatozoa were frequently

seen to pass by an egg so as almost to touch it, apparently without being in any way influenced by its presence. Nothing was seen which in any way reminded me of the chemotactic phenomena either of bacteria or of the spermatozoa of ferns.

It is undoubtedly true that the spermatozoa collect rapidly in the gelatinous coat of an egg. This is, however, due to the fact that the spermatozoa which strike the outer surface immediately bore into the interior. It will subsequently be shown more fully that the phenomenon takes place equally well when the jelly encloses (1) a ripe egg; (2) an egg not having undergone maturation; and (3) an egg which has been killed with osmic acid, and then washed. There is thus not the slightest necessity to account for the collection of the spermatozoa in the gelatinous coat by any chemotactic substance which diffuses through the jelly into the sea-water, and so attracts spermatozoa towards the egg.

If a substance causing attraction is really excreted by the eggs one should be able to collect it. On this assumption the following experiments were made.

A freshly obtained female *Arbacia* was cut open. The eggs, which were then extruded by the animal in dense masses from the oviducts, were collected in about 100 c.c. sea-water contained in a crystallising dish. As soon as the eggs had settled to the bottom, for the purpose of washing them, the water was nearly all removed by means of a pipette. Another 100 c.c. was then added, and so much again removed after the eggs had settled that the latter, very thickly placed together, formed a layer in about 1—3 mm. of the sea-water. Sufficient oxygen could thus be obtained for respiration. The eggs were left in the water from two to twelve hours, usually about six. At the end of this period the water was filtered, and the eggs thus removed. Capillary glass tubes, about 12 mm. long and 0.1—0.3 mm. internal diameter, and closed at one end, were then half filled with the water by means of an air-pump. The tubes were then introduced into a large open drop of sea-water, in which fresh, highly motile

spermatozoa were swimming. If the eggs excrete an attracting substance it was argued that it should be present in the tubes, and the spermatozoa should collect there.

In order to make certain that the eggs had remained normal during their stay in the sea-water, just before filtration some of the eggs were tested by artificial fertilisation. In all the experiments upon which reliance has been placed for results this took place in the normal manner, i. e. the vitelline membrane became raised. The first stages of segmentation were also often watched, and found in the majority of cases to follow the usual plan. The eggs were also tested soon after they had been placed in water. If, as rarely happened, they did not become fertilised readily they were rejected. The experiments were repeated with four sets (σ and ♀) of *Arbacia*, three of *Sphærechinus*, and two of *Echinus*.

No attraction of the spermatozoa into a tube could be observed. Except for a surface-contact phenomenon to be further discussed, they went in and out with indifference. Apparently, therefore, the water which had contained the eggs exercised no directive stimulus on the spermatozoa whatever.

I then attempted to find some substance which would give a chemotactic stimulus to the spermatozoa. The substances tested were such as are known to give a directive chemical stimulus to many protozoa, the spermatozoa of ferns, pollen-tubes, etc. The following solutions were tried by the capillary tube method: distilled water; meat extract 1 per cent.; potassium nitrate 10 per cent., 2 per cent.; sodium chloride 5·8 per cent., 2·9 per cent., 0·58 per cent.; potassium malate 1 per cent., 0·1 per cent.; asparagin 1 per cent.; glycerine 5 c.c. per cent.; grape sugar 18 per cent., 9 per cent., 4·5 per cent., 2·25 per cent.; peptone 1 per cent.; alcohol 50 per cent., 25 per cent., 10 per cent.; diastase (from Merk) 1 per cent.; oxalic acid 0·9 per cent., 0·09 per cent., 0·009 per cent.; nitric acid (concentrated) 1 per cent., 0·1 per cent., 0·01 per cent.

No definite chemotactic reaction—neither attraction nor repulsion—was observed in any case. Into tubes containing the weaker solutions the spermatozoa went in and out with apparent indifference. On coming into contact with highly concentrated neutral substances (potassium nitrate 10 per cent., sodium chloride 5·8 per cent., grape sugar 18 per cent.) the spermatozoa came to rest from loss of water. They are evidently not able to avoid solutions by a negative tonotactic reaction. On coming into contact with strong acid solutions (oxalic acid 0·9 per cent., 0·09 per cent., nitric acid 1 per cent., 0·1 per cent.) the spermatozoa were killed, and thus formed slight collections. They were thus not able to avoid acids by means of a negative chemotactic reaction.

Having obtained, so far as chemotaxis is concerned, only negative results by means of the capillary tube method, another method was employed, which Massart¹ found effective in determining the tonotactic sensibility of a number of marine micro-organisms.

Two large and equal drops were made in a moist chamber; one was of sea-water containing spermatozoa, the other distilled water. The drops were then joined by a narrow bridge, so that diffusion between them could take place. The experiment was watched between one and two hours. Some spermatozoa gradually entered the fresh water. No collection, however, took place in either drop. There were thus no signs of attraction or repulsion. The spermatozoa that entered the too diluted water were killed.

In another experiment a small drop of sea-water was dried upon a glass slide. A large oval drop of sea-water containing motile spermatozoa was then placed near and spread so that one end just covered the crystals from the dried drop. The crystals began to dissolve rapidly, locally concentrating the sea-water. As diffusion from the concentrated end of the drop took place the spermatozoa in the neighbourhood

¹ Massart, "Recherches sur les Organismes Inférieurs. II. La Sensibilité à la Concentration chez les Êtres Unicellulaires Marins," 'Bull. del 'Acad. roy. de Belgique,' 3me sér., tome xxii, No. 8, 1891, p. 148.

were killed or brought to rest. They were thus not repelled by concentrated sea-water.

When to one end of a large oval drop containing spermatozoa some crystals of sodium chloride or potassium nitrate were added, similar results were obtained. The spermatozoa allowed themselves to be surprised by the advancing salt and were accordingly killed.

The drop experiments were, then, not more successful than those with capillary tubes. No evidence of chemotactic reaction could be obtained by either method.

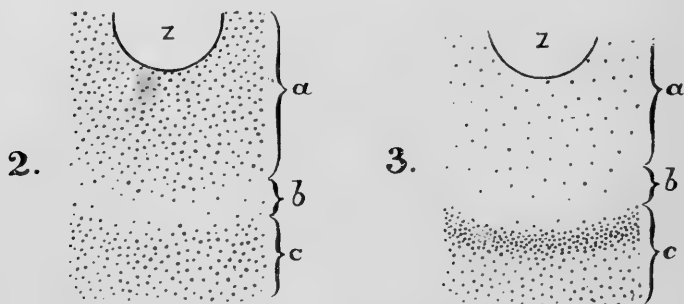
Massart¹ found by experiment that two species of *Spirillum* A and C, the flagellate *Heteromita rostrata* and two species of *Ciliata* fled from solutions both more or less concentrated than sea-water, i. e. they always sought a zone with the concentration equal to their normal medium. *Oxytricha gibba* also fled from solutions more highly concentrated than sea-water, but failed to avoid those less concentrated. In the case of his *Spirillum* B he obtained an organism which did not flee from solutions either more or less concentrated than sea-water, and which suffered in the experiments accordingly. Both the drop and capillary tube experiments described above appear to indicate that the spermatozoa of the Echinoidea, like Massart's *Spirillum* B, are quite insensible to tonotactic stimuli.

Finally, I attempted to determine whether the spermatozoa are attracted or repelled by oxygen. Fresh spermatozoa were removed from a testis and placed fairly thickly together in a drop, so that the latter was very slightly milky. A cover-glass 0.15 mm. thick and 18 mm. square, supported on pieces of another cover-glass also 0.15 mm. thick, was then placed upon the drop in such fashion as to include a small bubble of air near the middle. Under these circumstances one sees neither attraction nor repulsion from the bubble, even when the experiment has continued some time and when the oxygen supply must be getting low.

When, however, the drop is made very milky by spreading a

¹ Massart, loc. cit., pp. 151—154.

little of the thick white sperm-fluid in it by means of a pipette, a peculiar effect may be observed as a result of the presence of an air-bubble. The spermatozoa, in incredible numbers and constantly colliding, first swarm equally well all over the preparation. After about five minutes one sees macroscopically, when looking at the slide upon the microscope stage, a black zone arise about 1 mm. from the edge of the air-bubble. On examination with the microscope one sees that there are fewer spermatozoa there than anywhere else. Three zones (Fig. 2) may then be made out around the air-bubble *z*: *a*, an inner zone crowded with actively motile spermatozoa; *b*, a much thinner zone (that appearing macro-



FIGS. 2 and 3.

scopically black) in which there are comparatively very few spermatozoa; and *c*, the zone outside *b* (which extends over the rest of the preparation nearly to the edge of the cover-glass) where the spermatozoa are crowded, so far as I could judge, about as thickly as in zone *a*, but have all come to rest from want of oxygen. As one watches the preparation one sees (fig. 3) that the spermatozoa gradually leave the zone *a* and collect on the inner edge of the zone *c*, upon reaching which they cease to move. A ring of thickly placed, dead spermatozoa thus arises. A similar collection of dead spermatozoa also takes place about 1 mm. from the edge of the cover-glass. The explanation of this curious phenomenon,

which was observed many times for both *Arbacia* and *Echinus*, may be as follows:—The spermatozoa in the zone *c* after using up all the oxygen at once come to rest. The zones *a* and *b* are by diffusion supplied with oxygen from the bubble of air. The spermatozoa in these zones are thus able to continue in movement. This they do in any direction until reaching the inner edge of the zone *c*; when the oxygen has all been used up they can move no longer, and come to rest there, forming a ring. It is not, however, clear to me why the zone *b* should not be as crowded as the zone *a*. In any case there is no gathering of motile spermatozoa in any zone around the air-bubble. The phenomenon here described has then a totally different appearance to that figured by Massart¹ for the oxytaxis of *Spirillum* and of *Anophrys*, and is also quite unlike the collection of *Spirillum undula* around air-bubbles, which I myself have had frequent occasion to observe. I therefore fail to see in the phenomenon any evidence that the spermatozoa are attracted by oxygen.

From the above section of my paper it will be noticed that I have been unable to obtain any evidence, either direct or indirect, that the spermatozoa are attracted chemotactically to the eggs, and that, further, no success has attended my efforts to find any substance to which the spermatozoa are chemotactically sensible.

VI. THE MOVEMENTS OF SPERMATOCYTES UPON SURFACES.

When swimming in a drop of sea-water and not in contact with its surface the spermatozoa of the Echinoidea swim spirally. The spirals may be so steep that the spermatozoa appear to swim in almost a straight line, and they then move relatively rapidly across the field of the microscope. On the other hand, the incline of the spiral may be so gentle that the spermatozoa appear to be swimming almost in circles. In

¹ Massart, loc. cit., p. 157.

this case the progress across the microscopic field is very slow indeed. Between these extremes there is every gradation. An approximation to the first case appears, however, to be the rule with the most active spermatozoa.

When a spermatozoon comes in contact with a glass surface it is influenced by it in a curious manner. The spermatozoon remains in contact with the surface, and, unless it becomes immediately fixed to the glass, begins to make characteristic circular revolutions upon it. If the cover-glass be supported by pieces of another cover-glass, and the upper surface of the drop in contact with it be carefully focussed, it is seen that all the spermatozoa which are not attached by their heads but are moving there, are revolving, from the observer's point of view, in a clockwise direction. If the lower surface of the drop in contact with the slide be examined a reverse rotation—the counter-clockwise—is seen to be the rule. In both cases, therefore, if the surfaces be regarded from the point of view of the spermatozoa the rotation is always in one direction—namely, the counter-clockwise.

The clinging to surfaces and rotation upon them by the spermatozoa in the manner explained is not limited to glass. It takes place quite as well upon the surface of a drop bounded by air, and it is easily seen upon the outer surface of the gelatinous layer of eggs of *Echinus* which have just been placed in sea-water. I have also sometimes watched it (in the case of *Echinus*) upon the protoplasm of the eggs and upon the vitelline membrane, where it was made possible by the looseness of the zona pellucida. The nature of the surface for the phenomenon does not, therefore, appear to be of essential importance.

The following rule was found to hold good: whenever the spermatozoa of the Echinoidea come in contact with a surface they either become fixed to it at once or, more often, they rotate upon it, and in the latter case, looking from them to the surface in question, in a counter-clockwise direction.

The phenomenon is most easily seen in open drops containing not too many spermatozoa. The drops I employed

were about 10 mm. diameter and 1—2 mm. high. The upper and under surfaces (in which the direction of rotation appears reversed) are best examined with a magnification of from 70—240 diameters. In the case of *Echinus* the rotation is well seen under these conditions for ten minutes after the beginning of the experiment, fairly well for fifteen minutes, and ceases to be seen in about twenty minutes, when the spermatozoa have nearly all come to rest.

The rule given above as regards rotation upon surfaces was found to hold good not only for the Echinoidea, but for every group of the Echinodermata. Open drops were employed in the test. The names of the species examined have already been given.

It has already been mentioned that Dewitz¹ discovered that the spermatozoa of certain insects revolve upon surfaces. This has been confirmed by Ballowitz.² Dewitz also found that the direction of revolution was counter-clockwise. It is a remarkable fact that the spermatozoa of groups so far apart as the Insecta and Echinodermata should thus be affected by surfaces in the same manner. It is not improbable that a similar phenomenon will be found for the spermatozoa of yet other animals.

Immediately after my work had been brought to a close a preliminary paper was published by Dungere,³ entitled "Die Ursachen der Specietät bei der Befruchtung." In it the author communicated his discovery of the counter-clockwise rotation upon surfaces by the spermatozoa of *Sphærechinus* and *Arbacia*. He failed, however, to observe any rotation of the spermatozoa of *Echinus*, but this, he states, took place after the addition of certain (unnamed) "stimulating substances." Since it was in ordinary sea-water and with the spermatozoa of *Echinus* that I (independently) first became aware of the rotation, and since with this species

¹ Dewitz, loc. cit.

² Ballowitz, "Untersuchungen über die Structur der Spermatozoen, etc.," *Zeitschr. f. Zoologie*, Bd. I, 1890, pp. 392, 393.

³ Dungere, 'Centralbl. für Physiologie,' April, 1901, Heft 1.

I have made scores of observations upon the phenomenon in question, I am at a loss to explain his negative results. The detailed paper which he has promised will doubtless clear the matter up. At present, however, I am unable to accept his account of the "stimulating substances."

From the published figures and from my own observations the spermatozoa of the Echinoidea appear to be radial structures. The fact, however, that they rotate as a rule only in one direction shows that they cannot really be thus constructed. The researches of Ballowitz¹ upon bird and insect spermatozoa have demonstrated the complexity of their form, which is often in part spiral. Perhaps a minute investigation would also lead to similar results in the case of the Echinoidea.

Careful observation of the rotating spermatozoa reveals the fact that a few spermatozoa, probably not more than 1 per cent., revolve in a direction contrary to that of the great majority.

All that can be seen of a spermatozoon during its rotation upon the under side of a cover-glass is its head. This moves rapidly round in a circle. The tail is quite invisible. The centre of the circle remains fairly constant in position. Sometimes, however, the spermatozoon makes a wider curve for a while, and then begins to make circles with the same diameter as before around a new centre. The width of the circles varies. It appears, however, in the case of *Echinus* to be slightly less than 0.05 mm., the length of a spermatozoon. The rate of rotation of the actively moving spermatozoa in any preparation is fairly constant, as may easily be observed by direct comparison of rotating individuals. In the case of *Sphærechinus* a normally rotating spermatozoon was observed to make 109 circles around one point in 90 seconds, which gives a rate of slightly more than one revolution a second. The diameter of the circle is about the same as in the case of *Echinus*. Assuming it to be 0.04 mm., the rate of movement of the head is calculated to be approximately 0.12 mm. per second, or 7 mm. per minute.

If a supported cover-glass preparation containing a drop

¹ Ballowitz, loc. cit., p. 317.

with freshly obtained spermatozoa be made as quickly as possible and examined at once, one notices that a large number of spermatozoa are already rotating in the manner just described upon the upper and lower surfaces. One also notices, however, that a considerable number of spermatozoa have become attached by the ends of their conical heads. The heads then generally continue to move about their tips, usually executing circles apparently either clockwise or counter-clockwise.

If a single spermatozoon not fixed by the head and rotating in circles upon glass be watched, it will often be seen to make a number of consecutive revolutions and then suddenly stop in its course and become fixed to the glass by the point of its head. In this way the number of spermatozoa attached to the glass surfaces gradually increases.

Although the spermatozoa of the Echinoidea appear to become most easily fixed to surfaces by the tips of their conical heads, yet fixation may take place in several other ways. Thus, I have seen spermatozoa attached (1) by the middle of the side of the head, (2) by the middle-piece, proof of such fixation being rotation of the head around these respective parts. The spermatozoa can also become attached by the hinder half of the tail, for I have observed cases in which the head and fore part of the tail have made excursions from the glass, returning, however, to their original position, while the hind part of the tail remained in one place, immovable upon the glass surface. In yet other cases one sees a spermatozoon apparently attached to the glass by its whole length, slight waves of movement proceeding down the tail from just below the head. When spermatozoa have entirely come to rest upon a surface they are very frequently seen to be attached to the glass by their whole length, a fact which Ballowitz¹ has also observed in the case of the spermatozoa of Insects. From these various observations it appears that a spermatozoon may become attached to a surface in almost every possible manner.

¹ Ballowitz, loc. cit., p. 393, footnote.

One may not infrequently see a spermatozoon attached lengthwise to the under surface of a cover-glass move in a circle by means of a series of jerks, between every two of which it comes to rest. In these cases one often clearly sees the whole spermatozoon and not merely the head. One may then observe that the tail is curved and that circulation takes place in the direction of the curve. Fig. 4 represents what apparently happens, the spermatozoon being drawn in the resting stages. It is quite evident that rotation does not take place upon the tip of the tail, but that the whole tail at each jerk takes up a new position.

Observations upon a few (possibly nearly exhausted or immature) spermatozoa lying just beneath, and apparently attached to the surface of the cover-glass, showed that, as in the case of insects, the head does not alter its shape and is not concerned in locomotion, and that waves of movement arise in the fore-part of the tail and proceed toward its end. The driving force thus appears to lie close beneath the head.

With regard to the manner in which the rapid and continuous revolutions upon surfaces take place, if isolated observations upon a number of slowly moving spermatozoa will allow one to draw conclusions, perhaps the explanation may be as follows:—When a spermatozoon revolving in a spiral comes into accidental contact with a glass surface by the tail, at least the hinder end of this is unable to leave the glass owing to its adhesiveness, but can more or less easily be dragged along it. The fore part of the tail, by means of its automatic movement, causes the head and itself to make constant excursions to and from the glass surface. The tail is probably slightly curved, and the direction of motion of the spermatozoon is thus constrained to be circular. The head must frequently come in contact with the glass by its tip, which is specially adhesive and liable to become fixed in one place. Ballowitz holds that the circles upon surfaces for insects are probably simply the modified spiral turns of the free-swimming spermatozoa. This view, which

gives a purely mechanical explanation, is probably also correct for the spermatozoa of the Echinodermata. I therefore agree with Ballowitz¹ that Dewitz was in error in assuming that the movement of spermatozoa in circles upon surfaces is due to a special stimulus arising from the latter.

The Flagellata and Ciliata on meeting with an obstacle receive a mechanical stimulus,² to which they respond by reversing their direction of movement for a while. They thus avoid obstacles. It is evident from what has gone before that the spermatozoa of the Echinodermata do not react in this manner.

One may now inquire what significance the relations of sper-

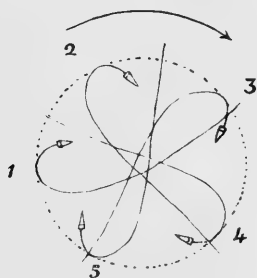


FIG. 4.

matozoa to surfaces has for fertilisation. Special observations to obtain an answer were made upon the eggs of *Echinus*. It was found that if spermatozoa be added to eggs which have just been placed in water, spermatozoa at once collect upon the outer surface of the gelatinous coat, and a number can be seen there making the characteristic circles. Others are seen to penetrate the jelly immediately on coming in contact with it. It is these spermatozoa which do not rotate upon the gelatinous coat which reach the living egg first. Of those spermatozoa which do rotate it was seen that they

¹ Ballowitz, loc. cit., p. 393.

² Jennings, "On the Movements and Motor Reflexes of the Flagellata and Ciliata," 'Amer. Journ. of Physiology,' vol. iii, Jan., 1900, p. 229.

often stop in their rotation, and that the head becomes fixed in the jelly. Sometimes the spermatozoon then succeeds in boring its way through, and may then reach the living protoplasm. In most cases, however, the head of a spermatozoon which has rotated a number of times gets stuck in the outer layer of the jelly, and no successful penetration occurs.

The gelatinous coat of an egg which has only been in water a few minutes is much more difficult for the spermatozoa to penetrate than that of an egg which has been in water several hours. Comparative experiments easily demonstrated this point, the difference being really striking. The jelly, as already mentioned, swells in water, and gradually nearly doubles its original breadth. At the same time it becomes softer. When spermatozoa are added after the swelling has taken place, scarcely a single spermatozoon is seen to rotate upon the eggs; on the contrary, they nearly all succeed in fixing their heads in the jelly, and the majority penetrate almost up to the living egg.

Dewitz¹ believes that the rotation of the spermatozoa of *Blatta* upon surfaces is of prime importance in enabling the spermatozoa to find their way into the micropyles of the eggs. This may well be the fact. In the case of the Echinoidea, however, there are no micropyles, and the gelatinous coat is everywhere penetrable. Further, rotation upon the eggs appears not to be the rule. It seems to me, therefore, that the fact that the spermatozoa will rotate upon resistant surfaces has no special biological significance in respect to fertilisation. On the other hand, the ability of the spermatozoa to cling to surfaces and to get stuck to them by the pointed end of their heads is of great importance in causing them not to leave the gelatinous coat of an egg after having come in contact with it, and in penetrating the same.

¹ Dewitz, loc. cit.

VII. THE DIRECTION OF PENETRATION OF THE GELATINOUS COAT.

Fol¹ observed for *Asterias* that when a spermatozoon had come in contact with the gelatinous coat it placed itself perpendicular to it, and then penetrated radially to the egg, meeting and fusing when half-way through with a curious "cone d'exudation." Fol concluded that the gelatinous coat is an apparatus for catching the spermatozoa when they come in contact with it, and attributed the radial structure to lines of more or less resistance, which serve to guide the spermatozoa directly to the egg.

Selenka² investigated the development of the gelatinous coat, and found that at first it is penetrated by fine radial protoplasmic filaments, each in its own canal. Later the filaments become withdrawn, but the canals remain until after fertilisation. Selenka also observed that the spermatozoa penetrate the jelly "always in a radial direction,"³ and stated that this is due to the spermatozoa making their way through the canals. This explanation appears to be very plausible. Before, however, accepting it as being sufficient, we shall do well to bear in mind the observations of Kupffer and Benecke upon the fertilisation of the eggs of *Petromyzon*.

According to the last-named authors the spermatozoa of *Petromyzon* penetrate a thick gelatinous dome covering one end of an egg in a radial direction.⁴ This observation, upon which special stress was laid, confirmed the statements previously made by August Müller. Although the inner shell-layer was found to contain radial canals, Kupffer and Benecke could discover no trace of such in the outer shell-layer, while they described and figured the dome as being

¹ Fol, 'Recherches sur la Fécondation, etc.,' 1879.

² Selenka, 'Zoologische Studien, Befruchtung des Eies von *Toxopneustes variegatus*,' 1878, p. 2.

³ Loc. cit., p. 5.

⁴ Kupffer and Benecke, 'Der Vorgang der Befruchtung am Ei der Neunaugen,' Königsberg, 1878, p. 11.

quite hyaline. The radial path of the spermatozoon is so striking that the authors believed it necessary to postulate some attraction¹ of the egg for the spermatozoon from a distance. Concerning the nature of the forces, however, no suggestion was made.

In my own investigations special attention was paid to the direction of penetration of the spermatozoa through the gelatinous coat of the eggs of *Echinus*. In this case, at least, it cannot be stated that penetration is always in a radial direction. A great many spermatozoa penetrate obliquely. It appeared to me, however, after having made a large number of observations for determining the point, that on the whole there is a tendency for the spermatozoa to make their way from the outside to the inside of the gelatinous coat. This tendency is best seen after the eggs have been from three to six hours in sea-water and the jelly has become considerably swollen. One then observes, upon adding spermatozoa, that on the whole, although many penetrate obliquely, the spermatozoa pass in a radial manner through the jelly to the egg. It is easy to observe spermatozoa which take an almost perfectly radial course. The path of many of them is seen to incline to a radius by an angle equal to between 10° and 30° . Others may be observed to start fairly radially, soon turn aside, and continue obliquely striking the eggs thus obliquely, or occasionally even making their way out again in a tangential direction. A considerable number of spermatozoa, after entering, stick fast in the jelly. The heads of these are then seen to be very variously oriented with respect to a radius.

Having come to the conclusion that the spermatozoa do pass more or less radially through the gelatinous coat, my next inquiry was concerning the cause. It was found that the radial penetration could be equally well observed in (1) a ripe egg; (2) a full-sized egg which had not undergone maturation, the nucleus being still very large and uncontracted; and in (3) a ripe egg which had been killed with

¹ Kupffer and Benecke, loc. cit., figs. 1, 7, and 8.

osmic acid and then washed. In the last case the osmic acid turned the eggs brown. The eggs so killed were put in 100 c.c. sea-water for half an hour and stirred round at intervals. They were then caught in a pipette, placed in a drop on a slide, and spermatozoa added. The radial penetration was quite as clear as in the living eggs.

From the foregoing observations it seems evident that the radial penetration is not brought about by any special attraction by the living egg, for it takes place equally well with a dead egg. Nor do the facts point to any chemotactic attracting substance as causing the phenomenon, for from a dead egg no excretion can take place. Selenka's suggestion that the spermatozoa take a radial course because they make their way through canals, which during the development of the egg contained protoplasmic connections, also does not seem to me a satisfactory explanation. The radial structure of the gelatinous coat after an egg has been a few hours in water is extremely faint, and, so far as one can directly observe, absent at the periphery where the spermatozoa start on their course. Selenka¹ admitted that the canals were finer than the width of the head of a spermatozoon. Surely with the swelling of the jelly these canals must be practically filled up. I have, as already stated, very frequently seen spermatozoa penetrate the gelatinous coat obliquely, often very obliquely. In these cases the spermatozoa could not be making their way through Selenka's canals. Hence we may conclude that the canals, if such there are, are not necessary for penetration. The thick gelatinous dome of a *Petromyzon* egg, and also, according to Massart,² the jelly around the ovum of the frog, are penetrated radially without the presence of any canals whatever. These various facts point to the conclusion that the penetration of the gelatinous coat in a more or less radial direction by the spermatozoa is not due to canals, but to some other cause.

The above reflections led me to make experiments to find

¹ Selenka, loc. cit., p. 5.

² Massart, "Sur la Pénétration, etc.," loc. cit., p. 217.

out how the spermatozoa behave toward jelly from other sources than that from the eggs of the Echinoidea.

When the oosporangia of *Cystocyra barbata* (one of the Fucaceæ) are liberated into sea-water, the outer coat rapidly swells and gelatinises. Spermatozoa from *Arbacia* were added to a preparation containing some of the oosporangia. At a certain stage in the gelatinisation the spermatozoa entered the jelly in large numbers, thus becoming densely crowded together in it.

A similar gathering was observed when the seed-coat of *Linum usatissimum* was placed in water containing spermatozoa. The outer cell-walls rapidly swell and become gelatinous. The spermatozoa, when the jelly had reached a certain consistency, collected in it in large numbers.

It was also found that if the gelatinous coat of an *Echinus* egg be separated by shaking, and spermatozoa be allowed access to the coat after several hours' isolation, the number of spermatozoa which will gather in it is very considerable.

The conclusion that is to be drawn from the above experiments appears to be that the spermatozoa are so constructed that they will bore their way into any jelly of a certain consistency without any aid from canals, chemotactic substances, or influences from living protoplasm.

Massart, as already mentioned, explains the radial penetration in the frog by supposing that the spermatozoa seek to pass from the more watery to the less watery layers of jelly, owing to a sensibility to these differences in saturation. Although this theory is plausible, it does not appear to me to be convincing. It does not sufficiently explain why the head of a spermatozoon is at first pushed into the jelly in a radial direction. After the head has been pushed in, whether this be radially or somewhat obliquely, the spermatozoon of *Echinus* usually takes a fairly straight course with respect to the axis of the head. Evidence of picking and choosing between the gelatinous layers thus appears to be wanting. After an egg of *Echinus* has been in water for several hours

it is doubtful whether the outer layers of jelly are the more watery and the inner the less so. In fact, from the ease with which the spermatozoa rotate the egg inside the gelatinous coat (*vide infra*), one might well suppose that the innermost layers are the more watery. The view that the resistance of the jelly decreases inwards has, indeed, already been upheld by Selenka¹ for the eggs of *Asterias*. For the *Echinoidea* and *Asteroidea*, therefore, the necessary basis of fact for an application of Massart's theory seems to be wanting.

There appear to me to be yet two possible explanations of the penetration: (1) It is due to reaction to a stereotactic stimulus; (2) it is purely mechanical.

1. Stereotropism has long been observed. Very many organisms, both animals and plants, in sea- and fresh-water, grow perpendicularly to their substratum, owing to the influence which the position of the latter has upon their direction of growth. In the same manner as for geotropism, heliotropism, chemotropism, etc., we have a corresponding tactic phenomenon, so also may it be with stereotropism. It is possible to imagine a free-swimming organism which, upon coming in contact with a surface, receives from it a stimulus which causes it to alter its movements in such a manner as to attempt to make its way more or less perpendicularly to the same, and through the substance concerned. Although such a stereotactic sensitiveness would neatly explain the radial penetration for the *Echinoidea*, *Petromyzon*, and the frog, yet conclusive observations in its support appear to me to be lacking.

2. Owing to the extreme difficulty or impossibility of seeing exactly what the movements of a spermatozoon upon a gelatinous surface are, the mechanical explanation must at present remain tentative and almost purely hypothetical. When a spermatozoon, swimming spirally, comes in contact with the outer surface of the gelatinous coat, the tip of the conical head, which reaches it first, possibly owing to the force of contact, possibly to adhesiveness, may well be supposed to

¹ Selenka, *loc. cit.*, II 'Die Befruchtung, Das Spermatozoon.'

immediately fix itself in the jelly. This is, indeed, what appears to take place under the microscope. The tail of the spermatozoon then probably adheres to the outer surface of the egg-coat, and is dragged round and round on it about the conical head, which is gradually pushed forward through the jelly. It may well be these revolutions (the modified spiral of the usual mode of swimming) which cause a spermatozoon to bore through the jelly more or less perpendicularly to the surface. The fact that the head is of such a shape that when once embedded in the gelatinous coat it can be easily pushed forward, but offers considerable resistance to moving either backwards or sideways, together with the particular consistency of the jelly, may well account for the steady progress forward of the whole spermatozoon in one direction. It seems to me probable that some such explanation as the foregoing will be sufficient to explain all that takes place during the penetration of the gelatinous coat.

It may here be remarked that since the presence of a gelatinous coat doubles the diameter of an egg its presence multiplies the chances of contact with its exterior surface by a spermatozoon four times. Since the more active spermatozoa, after coming in contact with the jelly, are conducted by it to the living protoplasm of the egg, the chances of fertilisation by them is, by the presence of the gelatinous coat, also increased four times. Since, however, a considerable percentage of the weaker spermatozoa get stuck in the gelatinous coat after entrance, thus not reaching the living egg, our estimate of the increased chances of fertilisation must undergo a large reduction. The first function of the jelly, which surrounds so many eggs, appears to be that of protection, making them distasteful to larger, and unassailable by smaller, enemies. For the purpose of fertilisation its consistency must be such as to allow easy penetration by the spermatozoa.

The ease with which spermatozoa enter and become fixed in gelatinous substances will explain a phenomenon which at first puzzled me. It was observed that, when a capillary

tube containing sea water, in which eggs had previously been deposited, was placed in a drop containing spermatozoa, the spermatozoa were not attracted into the tube. On the other hand, it frequently happened that the spermatozoa gathered very thickly into small balls just inside and outside of a tube. The balls were sometimes 0·01 to 0·05 mm. in diameter. It was apparent that, since the balls were only formed at the mouth of a tube, the cause of their formation was to be sought in the filtered sea-water. The phenomenon was found to take place after six successive filtrations. A drop of sea-water in which eggs had been deposited was placed upon a slide and a drop containing spermatozoa near it. On joining the drops a large number of small balls were formed in a very few seconds. When very numerous spermatozoa were present the balls became 0·1 mm. in diameter, containing many thousands of spermatozoa packed together in a dense mass. The following appears to be the explanation of the phenomenon:—From the ovary there come out with the eggs a large number of very small bits of jelly, which are so small that they will (like spermatozoa) pass through ordinary filter paper, and so transparent that one cannot directly see them. A few spermatozoa become attached to each piece of jelly, the presence of which may be inferred from the manner in which the small group of spermatozoa move about. Owing to the length of a spermatozoon, although its head may be embedded in a jelly particle, the tail may remain partly free. The little collections of spermatozoa thus move about hither and thither in no particular direction. When two such groups come by accident into contact they fuse. Certain of the spermatozoa adhere to both little masses of jelly and lock them together. The fused mass combines with other simple and fused masses, and so on. It is by this curious synthetic process that, in a very few seconds, there may be formed a ball as large or larger than an *Echinus* egg and containing thousands and thousands of spermatozoa, looking black under the microscope, and easily seen in a drop of water with the unaided eye.

VIII. THE ATTACHMENT OF SPERMATOOZOA TO THE EGG.

As soon as a spermatozoon has penetrated the gelatinous coat it usually becomes fixed by the head to the periphery of the living egg. Sometimes it executes circles for a while upon the protoplasm, and occasionally even re-enters the jelly and makes its way through this in a radial direction, thus leaving the egg entirely.

When a great number of spermatozoa are allowed access to an egg which has been some hours in sea-water, so many immediately penetrate and become attached by their heads that they set the egg in rotation. The rotation may be in any direction,¹ and often continues for about a minute, ceasing with the formation of the vitelline membrane. The rate of revolution varies according to the number of spermatozoa attached to the egg. A rapidly moving egg of *Arbacia* was observed to make ten revolutions in thirty seconds. The gelatinous coat during rotation scarcely moves at all, the living egg revolving quite independently within it. The spermatozoa often move the egg with such force as to separate it from its gelatinous coat. One then observes that, except for those attached by their heads to the egg, there is no collection of spermatozoa around the latter. This fact is in accordance with the supposition that no chemotactic substance is excreted by the egg. Numerous spermatozoa enter the isolated gelatinous coat.

The spermatozoon attaches itself to the egg by its most adhesive part, i. e. the tip of the head. The question arises whether the attachment is purely mechanical. It may be that the outer surface of the protoplasm is such as to be best adapted for retaining a spermatozoon by adhesion as

¹ For the eggs of the Fucaceæ the rotation appears to be constantly in a clockwise direction. Thus Farmer and Williams ('Phil. Trans. Roy. Soc.,' vol. 190, 1898, p. 633) state for *Halidrys* "the movement is always in a clockwise direction." I have also found this true for *Cystocyra barbata*. The fact as yet has not been explained.

soon as this comes in contact with it by the tip of its head. On the other hand, it is possible, and even probable, that unknown stimuli here play a part. The advance of the spermatozoon into the egg after leaving the periphery is, like the formation of the vitelline membrane, doubtless due to a stimulus given the egg by the spermatozoon. With regard to the exact nature of the stimulus and of the protoplasmic movements which appear to be its reaction we are as yet without any explanation.

IX. SUMMARY OF THE CHIEF RESULTS.

The chief conclusions arrived at during the research upon the fertilisation of the eggs of the Echinoidea were as follows :

1. The meeting of the spermatozoa with the outer surface of the gelatinous coat (zona pellucida) is a matter of chance, and not due to chemotaxis.

2. The passage of the spermatozoa through the gelatinous coat (observed chiefly in *Echinus*) is more or less in a radial direction as regards the egg. The direction taken is not due to any chemotactic substance being excreted from the egg. The phenomenon is possibly due to stereotaxis, but a purely mechanical explanation seems to the author more probable.

3. The spermatozoa are probably not chemotactically sensitive. They do not respond to tonotactic or heliotactic stimuli.

4. On coming in contact with a surface bounding their medium the spermatozoa cling to it, and usually continue for a time to revolve upon it in (from their point of view) a counter-clockwise direction. This statement applies to every group of the Echinodermata.

5. The spermatozoa easily become attached to glass and other surfaces by the tips of their conical heads. This phenomenon doubtless plays a rôle in causing the spermatozoa to bore through the gelatinous coat after having come in con-

tact with its outer surface, and also in their becoming attached to the living egg.

6. The vast number of eggs, and still vaster number of spermatozoa produced, together with the motility of the latter and the action of sea-currents, quite suffices to bring the male sexual cells into contact with the zona pellucida.

7. Many writers have supposed that chemotaxis is a constant factor in the fertilisation of animal eggs. This generalisation, which has been made by arguing from the attraction of the spermatozoa to the eggs of certain plants, is as yet entirely without experimental justification. From my own results with the Echinoidea, which are in accordance with those obtained by Massart in the case of the frog, and with the work of Dewitz upon the fertilisation of the eggs of certain insects, I have been led to suppose that chemotaxis, at least for a great number of animal species, plays no rôle whatever in bringing the sexual elements together.

The work for the above paper was done at the Stazione Zoologica, Naples, during the months of March and April of each of the years 1900 and 1901. It gives me much pleasure to thank the Committee of the British Association for granting me the use of the table, and also to acknowledge my indebtedness to the staff of the Stazione Zoologica for supplying me with material and apparatus during the research.

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By E. RAY LANKESTER, M.A., LL.D., F.R.S.

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Maturation of the Ovum in *Echinus* *esculentus*.

By

Thomas H. Bryce, M.A., M.D.

With Plates 10—12.

INTRODUCTION.

THE subject of the maturation of the sexual cells is a thorny terrain. It can be attacked only by the highest powers of the microscope, and the facts can only be reached by a process of patient mental reconstruction of the various phases. Historically the subject has been overlaid by some brilliant but premature hypotheses, which, however much they may have stimulated research, have also tended to foster prepossessions. The necessary stimulus for research has been supplied by the hypothesis that the chromatin of the nucleus is the hereditary substance, or, at least, the bearer from one generation to another of hereditary qualities. But apart from the interest connected with problems of heredity, and the meaning of fertilisation, the study of the intricate details of the process of maturation goes to the bottom of all our knowledge of cellular morphology. The study of the maturation phenomena in *Echinus* was, in the first place, taken up merely with the motive of seeing some of the actual phases in the most readily obtainable material. But it was soon discovered that although the outward phases had been frequently studied, most of the finer details of the process, as seen in *Echinus*, were undescribed, and therefore it was considered worth while to make a study of the whole process.

In view of the hopelessly diverging results for different forms obtained by different observers, an interest in the behaviour of the chromatin during maturation has declined of recent years, and the question of the centrosome has occupied more attention. Results which came out led me to certain conclusions, which, to my mind, tended to clear up in some measure the confusion at present prevailing. As the research was proceeding, Strasburger's work, 'Reduktionstheilung, Spindelbildung, Centrosomen, und Cilien-bilden in Pflanzenreich' (1900), came into my hands. In that work conclusions in the matter of the reducing divisions identical with my own, and foreshadowed in several previous botanical memoirs, are brought, by new comparative investigations, to a focus, and are made a means of harmonising the apparently contradictory results in the case of plants. This obviously increased the importance of my own results, and inspired me to follow out, in spite of the large amount of labour involved, the whole series of phenomena, in order to obtain as complete a demonstration of the facts as possible.

MATURATION IN *ECHINUS ESCULENTUS*, L.

Previous Observations on Maturation in Echinoderms.

The Echinoderm ovum has been the classical material for all observations on the living egg. The earliest observations on the maturation of the sea-urchin egg were made by Derbes in 1847. Agassiz, in 1864, described the polar bodies in both *Toxopneustes* and *Asteracanthion*. Between 1872 and 1882 Van Beneden examined the phenomena in *Asterias*, Hertwig in *Toxopneustus lividus* and *Asteracanthion*, Giard in *Psammechinus*, Fol in *Asterias glacialis*, Greeff in *Asterias rubens*, and Flemming in *Spærechinus brevispinosus*, *Echinus miliaris*, and *Toxopneustes*. Since then the favourite material for the examination of the phenomena in the

living egg has been *Asterias*. Notwithstanding this, little is known as to the finer details of maturation. Hartmann (1902) has, since this paper was written, published an account of the changes in this egg up to the formation of the first polar spindle. The early observations were made on the entire egg—either in the living state, or fixed and cleared. The polar bodies in *Echinus* are normally thrown off within the ovary, and when the naked eggs are shed into the sea water they remain entangled in the connective tissue of that organ. Sometimes it may happen that a partially immature ovary may be manipulated and some ova caught in the maturation stages. In the starfish, on the other hand, the eggs commence to show the phases when placed in sea water, and they can be watched. Again, by shaking immature sea-urchin eggs the stages can be induced artificially. Boveri (1890) has figured a few stages after the formation of the first polar spindle in *Echinus microtuberculatus*, but either the chromosomes, which are very minute in *Echinus sphæra*, are still more minute in *Echinus microtuberculatus*, and cannot be further analysed, or he has not seen the figures which I have made out by my methods. Further, the number of chromosomes is different. Matthews (1895) examined maturation in *Asterias Forbesii*. He was able to obtain only one ovary showing the stages up to the formation of the first polar spindle, but supplemented his observations by stages obtained by shaking the eggs. He describes the behaviour of the centrosomes, but gives no details as to the chromatin. Wilson, in his atlas of 'Fertilisation and Karyokinesis,' shows a single photograph of a second polar spindle in *Toxopneustes*, and Boveri has drawn a single figure of the second polar spindle in his recent work published in 1901. Haecker (1893) also gives a diagrammatic drawing of the first polar spindle, but gives no description of maturation. In none of these figures is the finer constitution of the chromatin elements represented. Cuénot and other observers have written on oogenesis in Echinoderms,

but their observations were confined strictly to the ovary and the formation of its epithelium, and to certain points in the characters of the nucleolus. Various observers have treated specially of Echinoderm spermatogenesis (Jensen, Pictet, and others), and many have studied the morphology of the spermatozoon, but of these Field (1895) brings the latest account. Owing to the excessive minuteness of the chromosomes he seems to have confined himself to counting them in the different phases. Haecker (1893) published observations carried out on the living egg on the germinal vesicle and nucleolus of Echinoderms, but does not give any detail regarding maturation.

Personal Observations.

Methods.—My material was obtained from animals freshly out of the water.¹ Small pieces of close on seventy ovaries were fixed, embedded in paraffin, and a few dozen sections cut from each. These were all carefully examined, and when maturation was found to be proceeding some hundreds of sections were cut and gone over, and the details built up from these. The fixative fluids used were Flemming's strong solution, and Hermann's platinic chloride and osmic acid mixture; almost identical results were obtained by both, and the small pieces of ovary—about a cubic centimetre or a little more—were well fixed throughout. At a later stage of the research, by way of control, pieces of ovary were fixed in Boveri's picric and acetic acid mixture, and sublumo-acetic acid, as well as Lindsay Johnstone's fluid. The picro-acetic material was unsatisfactory, but the sublimate gave good results in some respects. The chromatin was, however, much better differentiated by the osmic acid mixtures, especially by Hermann's fluid; while in

¹ The material was obtained at the Marine Biological Station at Millport in late March and early April. At the end of April and beginning of May the ovaries are mature throughout. In January maturation has already begun, and from that time onward the relative proportion of mature to immature ova gradually increases.

the sublimate material the centrosome gave quite a different picture, as will be seen in the sequel.

Staining.—Osmic acid preparations being proverbially refractory to most staining reagents I have confined myself almost entirely to Heidenhain's iron hæmatoxylin method, but have used by way of control other stains. To facilitate staining I have always allowed my preparations to stand for some time in old turpentine to remove the osmic acid. The best results were obtained by iron hæmatoxylin alone, the picture presenting the vivid black chromosomes on a blue-grey field. Heidenhain's preliminary stain with Bordeaux red rather confuses the picture of the chromatin, and the only other contrast stain used was a very weak coloration by alcoholic solution of fuchsin S.

It is necessary here to refer to the recent criticism by Boveri (1901) of the iron hæmatoxylin stain. He shows, as every one knows who has used the method, that different degrees of washing out yield different results, and refers to the fact that structures may appear which owe their existence to a purely mechanical cause and not to any difference in chemical composition. Thus a part which is not readily accessible, on account of its position, to the differentiating fluid retains the stain while the parts in the neighbourhood are decolourised; further, the fluid having a concentric effect in washing out, the superficial parts are decolourised while the central parts retain the black stain. Thus he explains the different accounts given of the structure of the centrosome, and points out that by strong extraction of the colour even the chromosomes may be apparently diminished in size owing to their peripheral parts being decolourised. This is weighty criticism in view of a number of the appearances I shall have to describe, for he combats the generally accepted view that the true appearances are obtained by strong washing out, and believes that in regard to the centrosomes the opposite is true. As to the chromosomes I may forestall criticism of my results by stating, first, that I have obtained similar appearances both with the osmic

acid and the sublimate mixtures, and by other stains besides the iron hæmatoxylin, though the greatest vividness of differentiation has been obtained by a combination of Hermann's or Flemming's fluid with iron hæmatoxylin, and, second, that the proof that I am dealing with realities and not illusions is to be found in the fact that the appearances described for the chromosomes represent a complete and unbroken series of the steps or stages of a process that can be explained only by reference to the completed story.

The drawings were made by aid of the Abbe drawing apparatus of Zeiss, the finer detail being filled in free-hand. The combination used was in every case Zeiss 2 mm. 1.40 numerical aperture, apochromatic objective, with either eight or twelve compensating eye-piece. The illuminating apparatus employed was a Zeiss 1 mm. numerical aperture, achromatic condenser. The sections were cut in paraffin, and were of varying thickness. The object in most cases being to obtain the masses of chromatin entire, comparatively thick sections were taken, six to seven microns. Thinner sections down to three microns were employed to determine certain points regarding the achromatic structures.

In dealing with the subject I shall first describe the changes in the ovum leading up to the disappearance of the germinal vesicle, and after that treat in separate sections of the behaviour of the achromatic and of the chromatic structures.

My earliest preparations are from the growth period. Out of a large number of young oocytes of the first order I have only seen two or three in mitotic division, and these only in the spireme stage, so that I cannot speak as to the number of chromosomes in these divisions. The young ovum shows a delicate reticular protoplasmic structure (fig. 1). The nucleus is already large and vesicular, with a distinct nuclear membrane, and a deeply staining eccentric nucleolus. This being intensely black, contrasts strongly with the granular and irregular nuclear network, which refuses to take on the chromatin stain, and remains pink in prepara-

tions stained either with "fuchsin S" or "Bordeaux red." There is frequently a second smaller deeply staining circular body in the nucleus, but it has no regularity in position and is not invariably present. I cannot in any of my preparations see the double nature of the threads described by Haecker. Close to the nucleus, very frequently on the side of that body towards which the nucleolus lies, there is sometimes at this stage a body which presents much the appearance of the centrosome of a resting cell. It consists of either a single granule or pair of granules, sometimes a group of smaller granules enclosed in a circular area. While this may represent a centrosome it is impossible to distinguish it from similar bodies with central granules that may be found in other parts of the cell, which are undoubtedly cell inclusions, and therefore no structure can with certainty be identified as a centrosome.

Structure of the Protoplasm.—Wilson (1899) has shown that in the young ovum the protoplasm is granular, and that as the ovum grows in size an alveolar structure is assumed. In the youngest ova of my fixed material the protoplasm presents a granular appearance which is certainly not alveolar, and can hardly be termed reticular (fig. 1). In the fully grown egg the appearances vary according to the stain. In fig. 3 the cytoplasm is represented as showing a reticulum which is composed of separate minute granules; the meshes of this reticulum bound alveolar spaces. These alveoli are on the whole rounded, and in this particular specimen, from which the iron hæmatoxylin was very thoroughly washed out and replaced by a slight counter-stain by fuchsin, they were faintly red with a slightly darker periphery. In fig. 2, on the other hand, the appearances are different. The iron hæmatoxylin has not been so completely washed out, and the alveoli have retained the dark stain, showing up as rounded dark points separated by an unstained reticulum. Sometimes the centre of the alveolus is occupied by a black dot, as if the centre had not been decolourised. Thus my preparations fully bear out Wilson's

latest conclusion (1899) regarding the structure of the sea-urchin egg—namely, that the condition of the cytoplasm conforms to Bütschli's description. It has the same physical characters as an emulsion; that is, there is a fluid framework in which the microsomes are suspended, and the alveoli are filled with a fluid of different physical characters. When the alveoli are wholly destained all that is seen is the micro-somic network, whereas when they are stained the alveoli stand out as the yolk granules embedded in the cytoplasm. Wilson shows, however, that the cytoplasm at certain periods may have a fibrillar structure, but to this point I shall return later.

The changes which the nucleus undergoes during the growth of the oocyte, until it becomes the fully developed germinal vesicle, are very complicated and uncertain. Many irregular figures suggest that the germinal vesicle may undergo changes of shape. They may well be artefacts. I shall only refer to certain facts regarding the chemical reaction of the nucleus, which seem to be fully vouched for in my preparations. It has been shown by a number of observers that the staining reactions of the nucleus vary at different times. At one time the chromatin network will take the specific stains deeply, while at other times it remains unstained (Rückert, 1892). My experience tends to support these statements, though one must admit that very different effects are produced by different degrees of coloration with iron hæmatoxylin. The effect depends on the degree of extraction of the colour, but it is quite certain that at certain stages of the nucleus the network very readily parts with the black stain, and is left as an irregular granular reticulum of a blue-grey colour, or of a red tint, in preparations stained for contrast with rubin. The nucleolus, on the other hand, is exceedingly tenacious of the stain, and appears as an intensely black spot (fig. 2). Again, at a stage I consider to be of later date, the network shows a basis of delicate linin threads, with deeply stained chromatin particles arranged on the thread, giving it a very

irregular or feathery structure, while the nucleolus generally is less deeply stained and vacuolated (fig. 2). Finally, when the nucleus is fully grown and maturation imminent, we find the contrast is exactly the opposite of that described for the young nucleus. The network is intensely black, consisting of particles of chromatin arranged in a very intricate and irregular fashion, while the nucleolus parts with the stain very readily, and is left as an almost colourless, apparently empty vesicle. Soon after the resolution of the nuclear membrane it disappears from view. Very similar changes are described in many other forms,—for instance, in the Turbellarians, according to Francotte (1897); in *Polychærus*, according to Gardiner (1898); and according to Gathy (1900), in *Tubifex* (an Annelid) the nucleolus loses its capacity for staining with iron hæmatoxylin at the end of the growth period.

It is difficult to resist the conclusion that the chromatin substance is at first confined to the nucleolus, and later leaves it to form the chromatic basis of the nuclear network as a whole, and therefore also of the future chromosomes. The fate of the nucleolus in Echinoderm eggs has been variously interpreted. Derbes (in 1847) thought it was directly converted into the pronucleus of the mature egg, and Hertwig (1877) took the same view. Fol (1877) and Flemming (1882), however, proved that the chromatin itself became the future nucleus, after it was provided with a new nuclear membrane. Recently Carnoy and Le Brun (1899) have maintained the view that in the amphibian egg where there is no chief nucleolus, but a large number of smaller ones, certain of these become converted into the future chromosomes, thus reverting to the older view of Schultze (1887). It seems certainly true, as said above, that the chemical substance which is lodged in the nucleolus in the early ovum becomes later distributed into the germinal vesicle, and so indirectly goes to form the chromosomes. Hartmann (1902), for *Asterias glacialis*, describes the chromosomes passing directly out of the nucleolus, the remainder of the nuclear reticulum being rejected.

Strasburger regards the body as a storehouse of reserve substances, which pass into the cell during division to form the "kinoplasm," which goes to form the spindle, the Hautschicht, membranes, and cilia. We shall see later that the phenomena observed in the sea-urchin egg may combine these two views. But in contradiction to both is Haecker's view. His observations on the living egg of the sea-urchin reveal to him the nucleolus as a pulsating organ in which, periodically through the whole growth period, small vacuoles appear; these run into a single central vacuole, which increases and then diminishes in size. When the largest central vacuole appears the nucleolus removes itself to the periphery of the nucleus, and meantime the vacuole comes into relation with the outer layers of the nucleolus, as if to bring its contents into relation with the nuclear sap; and further, an indrawing of the wall of the germinal vesicle itself suggested that there was a communication between the cytoplasm and nucleolus. From these and other observations Haecker regards the nucleolus as a secretory organ, collecting the by-products of nuclear activity—not as a storehouse, or "nuclein laboratorium" (Fick, 1899). So far as my observations go, they tend to support the idea of the nucleolus being a storehouse or laboratory of nuclein.

Centrosome.—There has been a great deal of discussion as to this enigmatical structure in the sea-urchin egg. Varying accounts have emanated from Boveri, Wilson, Fol, Bütschli, Reinke, Hill, Kostanecki, and Erlanger. Boveri says, "Das Seeigel-Ei ist von allen objecten die von mir bekannt sind, dasjenige, welches einer sicheren Darstellung der Centrosomen die grossten Schwierigkeiten bereitet." This quotation is taken from his recent work, 'On the Nature of Centrosomes.' He reconciles more or less the different accounts, and suggests a nomenclature which I shall adopt as being the latest and most authoritative.

The centrosome is composed of a special and peculiar substance, the centroplasma, which, according to the perfec-

tion of fixation and the manner of staining, presents different appearances. This accounts for the different forms under which the body has appeared. It stains best with iron hæmatoxylin, and destains concentrically. When destaining has been carried far, it shows as a discoidal area surrounded by a clear halo, and has a very fine alveolar structure. This is the form in which I have observed it in all my osmic acid preparations, except that I do not see the halo, and when counterstained with rubin it has a red colour, which indefinitely fades away into the bluish-grey astral rays and spindle fibres. This rounded body, as division proceeds, becomes enlarged, then lens-shaped, and ultimately flattens into a plate which lies along the side of the nucleus. In polar view this is dumbbell-shaped; the enlarged ends are the daughter centrosomes which become surrounded with new radiations. In the maturation stages my preparations are not numerous enough to enable me to follow in detail the behaviour of the centrosomes, and I have not been fortunate enough to see the division of the body in the first maturation spindle. The centrosome of Boveri corresponds to the centrosphere of Wilson. In another set of preparations less destained, Boveri described the centrosome as a smaller body, showing in its centre a darkly staining particle, the centriole, which corresponds to Wilson's centrosome. This, as division proceeds, divides into two, and goes through the usually described evolutions. In picro-acetic and sublumo-acetic preparations I have seen such a centriole, but have been unable to trace its division. Again, when destaining has been stopped early the whole centropiasm is black. This I have also seen in picro-acetic and sublumo-acetic material. The rays, according to Boveri, stop at the margin of his centrosome, and do not enter it so as to be inserted into the centriole. This seems to be the case, and in my cleavage preparations fixed with Lindsay Johnstone's fluid the central reticular body is sometimes seen to have completely dropped out, so that the astral rays are seen to end abruptly, leaving an absolutely round empty space occupied in the other eggs by

the alveolar or reticular centropiasm. I do not presume to give an opinion on the much vexed question of the persistence of the centrosome as a special cell organ, but one thing seems clear, that the centropiasm is a focus of protoplasmic activity, and is ultimately to be explained on physiological and not on mechanical grounds.

Changes in the Germinal Vesicle Preparatory to Division.—When the germinal vesicle has reached its full growth the nucleolus loses its staining capacity to chromatin stains, the nuclear network takes an intense stain, and the cytoplasm to its very outer edge is seen to have an alveolar structure. In many cases, presumably in stages close to the onset of maturation, the nuclear membrane is puckered. The germinal vesicle then moves towards the surface, and, as long ago described by Hertwig (1877) for *Asteracanthion*, at the spot nearest the surface a protoplasmic process projects into its interior (fig. 4). In osmic acid preparations the nuclear membrane is seen to be indented and folded before the process; this, as it projects inwards, spreads out in every direction from the neck, so that at the margins of the process are seen sections of peninsulæ and islands. In sublimate material the nuclear membrane is not so sharply differentiated, and the inward folding of it is not so clearly seen. I have no doubt from my sections, such as shown in fig. 4, that this is a true invagination of the germinal vesicle by the cytoplasm. At the neck of the invagination the alveolar walls of the cytoplasm are drawn inwards towards the centre, but in the process itself no very distinct fibrillar structure is at first to be seen.

Hartmann represents at this stage a very distinct aster between the invaginated wall of the vesicle and the surface of the ovum. I have not seen such an aster in my sections; the wall of the vesicle is always very close to the surface of the egg, leaving no room for such a formation, and the aster seems to form within the process. Sometimes the radiations from the neck of the invagination are much better marked than in the ovum represented, and in the process itself there

is a distinct suggestion of radiation, which is, however, very difficult actually to define. The appearances suggest that the centrosome or kinetic centre lies in the neck of the invagination; but at this stage there is not, so far as the study from sections can determine, any wide-spreading astral formation as in *Asterias*. Soon it is seen that the whole mass is made up of what seem to be looping fibres. Possibly the folded and puckered nuclear membrane contributes to this appearance. In fig. 5 is represented a stage in which the chromatic reticulum has become finer, and at the neck of the process is an irregular mass which is destined to form the future chromosomes. All this time the germinal vesicle remains close to the periphery of the egg.

The next stage I can determine is the one represented in fig. 6. The nuclear membrane has now entirely disappeared, and in the irregular mass of looping fibres there are seen two asters. In each is a circular finely reticular area, the centrosplasm, and from the periphery pass out in every direction very delicate interdigitating fibres. Between the two asters the fibres are drawn out to form an irregular spindle arrangement. Round this area the greater part of the nuclear reticulum, which does not form chromosomes, but was related to the vegetative stage of the germinal vesicle, is seen merging with the cytoplasm, but still retaining its reticular character. Between the spindle and the surface the chromosomal chromatin mass is seen.

This description corresponds with Hertwig's original account, and also in the main with Hartmann's recent representation of the facts, but differs from Fol's in that he describes no process projecting into the vesicle. It also differs from Mathews' description of what occurs in *Asterias Forbesii*. He describes the two centrosomes probably passing out of the germinal vesicle at the nearest point to the surface of the egg by the rupture of the nuclear membrane at that point. They then pass some distance from the nucleus, and are seen to have round them a faint halo of "archoplasm." This latter becomes distinct, radiations are developed, the whole

archoplasmic area divides, and the two parts being drawn asunder, a spindle is spun out between them, which moves tangentially over the nucleus. As it grows the spindle-fibres project into the vesicle, the nuclear membrane is dissolved, and the spindle then rotates to become the first polar amphiaster. In Haecker's text-book (p. 123) the process in the living egg is described in much the same fashion. A clear area is developed between the remains of the germinal vesicle and the surface, surrounded by a radiation which soon forms a double star, which is the beginning of the amphiaster.

The invagination of the germinal vesicle in the egg of *Echinus* is probably secondary. It may be related to the fact that the wall of the vesicle comes exceedingly close to the surface of the egg. The mounting of the vesicle to the surface is a fact which, so far as I know, has not been satisfactorily explained. Haecker (1893) suggested that it is due to the action of gravity causing a movement in the elements of the egg after the force connected with the exchange of material between nucleus and cytoplasm, which keeps the vesicle in the centre of the egg, ceases with full growth. My preparations do not throw any light on the point.

Fig. 8 represents a somewhat oblique section of the germinal vesicle at a later stage. It shows the two asters arranged tangentially to the surface of the egg, but between them, and extending towards the surface, is a finely reticular mass, out of which the delicate wavy and interdigitating rays of the asters are evidently spun. Embedded in this reticulum are seen the chromatin segments. At a later stage (fig. 9) all these are drawn into the area between the asters, which is seen now as a finely alveolar or reticular plate. Round this central plate is a complicated reticulum of fibres crossing and intercrossing, but on the whole radiating from the central plate. In this reticular zone is also seen, at a little distance from the plate, one centrosome surrounded by rays, obviously part of the general reticulum. In the adjoining section a second aster was present on the side of the plate removed from the surface of the ovum. At this stage one hardly ever

sees a preparation in which both asters are cut in the same section. Griffin, in *Thalassema*, has described a disappearance of the spindle spun out between the centrosomes, and the development of a central mass very like that which I have described between the asters. In most instances, however, the conditions are more like those described by Matthews in *Asterias Forbesii*.

It is evident that when the nuclear membrane disappears, and the rejected chromatin passes into the cytoplasm, a profound effect is produced on the organisation of the egg. Whereas, with the germinal vesicle still intact, the alveolar structure can be traced to the surface of the egg, we now find that round the transformed nucleus, and projecting into the centre of the egg, is a fibrillar mass, which is sharply differentiated from the alveolar yolk. From this central area there also extends round the surface of the egg a layer of differentiated protoplasm. The central mass and surface layer have each a definite fate. The one is differentiated into the spindle and asters, while the surface layer is, I believe, associated with the formation of the membrane thrown off by the egg at the moment of fertilisation. The central mass of the yolk is unchanged in appearance, and the question is whether this reticular mass of protoplasm is differentiated from the cytoplasm, or is derived from the rejected nuclear reticulum. I am inclined to think that it is in large measure formed from, or under the influence of, the discarded nuclear material. This would be in harmony with the results of Carnoy and Le Brun (1899) in *Triton*. Another evidence of the excitement produced in the egg at this stage may perhaps be seen in the accessory asters formed, which, so far as I can see, have no relation to the formation of the definite asters of the spindle.

From experiments by R. Hertwig (1896) and Morgan (1896) it seems that under special artificial chemical stimulus the cytoplasm may be excited to form asters, and even, in Hertwig's experiments, amphiasters. Reinke (1894) also found that in the peritoneal cells of the larval salamander

three grades of asters are formed—primary, secondary, and tertiary. The last contribute to the secondary, and these again to the primary or definitive asters. Carnoy described accessory asters during the formation of the second polar body in *Ascaris*, and Meade (1897) showed that a great number of such asters were formed before the formation of the first polar spindle in *Chaetopterus* (an Annelid), which he thought contributed to the formation of the spindle asters. Watase (in *Macrobdella*) found as many as thirteen asters in the cytoplasm, with centres varying in size from the smallest microsome to the true centrosome. Griffin (1899) also describes the formation of accessory asters in *Thalassema*. These experiments and observations are held to afford strong evidence of the free formation of the centrosome, in which case both that body and its aster would be the expression rather than the cause of cell activities.

The secondary asters in *Echinus* at this stage are possibly produced in the cytoplasm under the influence of the nuclear material let loose on the disappearance of the nuclear membrane.

All this tallies better with Strasburger's views of the kinoplasm than with any other theory. He thinks of protoplasm as of two kinds, trophoplasm and kinoplasm: the former is vegetative in function and alveolar in structure; the latter presides over the activities of the cell, forms centrosomes, mid-bodies, asters, and spindles, constitutes a peripheral layer from which membranes and cilia are derived, and is fibrillar in structure. This differentiation of the protoplasm takes place when mitosis sets in. Further, he thinks the nucleolus is a storehouse of reserve material, out of which, on need, the substance of the kinoplasm is drawn.

I have shown that the nucleolus at first seems to contain all the chromatin substance which later is found in the nuclear reticulum, the larger portion of which is rejected, to form in turn, if I be right, directly or indirectly a reticular zone, out of which the asters and spindle are spun,

My conception of the meaning of the changes in the ovum does not, however, involve an acceptance of either Strasburger's kinoplasmic or of Boveri's archoplasmic theory. It inclines rather to the view that the same ground substance, under the influence of the chemical changes underlying vital activities, may take on different forms in response to varying physiological needs, and further, that whereas, during the vegetative period, the main centre of these chemical activities lies in the nucleolus, in the division period that centre is transferred to the centrosome, which is the expression of activities resulting in the vital phenomena of division.

Fig. 15 represents a later stage. The spindle is not yet complete, but the two asters are situated radially, and the reticular mass, though still showing in some parts a radial distribution from the central plate, is becoming more and more focussed on the centrosomes. The spindle, in most forms, is said to be fully formed before this radial position is assumed, and the whole spindle is said to rotate through 90 degrees. A very good example of this is seen in the egg of the mouse, as described by Sobotta (1895).

In my preparations the spindle, as is the case also in *Thalassema* (Griffin), is late in being completed, and the asters seem to move independently through the cytoplasm, the fibres arranging themselves round the centre of activity until the definitive position is reached.

The conditions described for the formation of the polar spindle are not unlike those accompanying the formation of the multipolar spindles described in the pollen and the spore-mother cells in many plants by Farmer, Belajeff, Osterhout, Mottier, Nemec, and Byxbee. According to the description of these authors there is a filar zone round the nucleus, out of which the multipolar figure is spun, the poles of which draw together to form the definitive bipolar spindle. I have seen one or two four-poled first-maturation spindles, but I cannot make out that in the reticular zone there are more than two asters which have any relation to the future spindles, and such four-poled spindles would thus merely indicate the

tendency to the formation of multiple centres of activity, or putting it in terms of the centrosome, to the formation of four centrosomes instead of two.

The whole process leading up to the formation of the first polar amphiaster is very complicated in *Echinus*, and extremely difficult to trace in sections. It is as difficult to be sure of the phases in the living *Echinus* egg, which are moreover difficult to get as the process normally takes place within the ovary. The process does not seem to me to be so simple as it has been described for *Asterias*; indeed, it is in many respects like what Mead has described for *Chætopterus*. Dr. Teacher has recently studied the phases in the living egg, and has kindly let me see his results, which supplement my own. He has seen frequently a stage which I have described as follows—"near the surface of the ovum is a clear granular area having the appearance of ground glass, surrounded by a darker ring merging into the alveolar-looking cytoplasm. This ring, at its circumference, is distinctly irregular, and suggests delicate radiations from the central granular area." This is obviously the stage represented by the section depicted in fig. 9, and corresponds to the transformed germinal vesicle. At this stage I could not make out distinct asters in the living egg, and in the sections the astral rays which lie within this area are of great delicacy. Dr. Teacher has seen in this phase many specimens with a number of asters in the cytoplasm around the transformed germinal vesicle, and has made out at the same time, within the area itself, astral formations which he believed to be the definitive asters of the spindle. While, therefore, I have in the foregoing description traced the centrosomes as if they were persistent centres travelling through the cytoplasm, I cannot exclude the possibility of their free formation as described by Mead.

Fig. 16 represents the spindle now completed. The chromosomes are being drawn into the equatorial plate. Fig. 17 shows the now completed spindle in metaphase. It is relatively bulky, with blunt and rounded ends, and

frequently, when the section is through the side of it, the centrosome is not cut at either end. There is no central spindle. The chromosomes extend through the whole equatorial plate, and the peripheral rays of the asters are seen interdigitating opposite the equator. The fibres of the spindle itself are somewhat uneven, and in relation to the chromosomes there are darker bundles apparently of several fibres spun together. As metakinesis proceeds the waviness of the fibres becomes more distinct. The central centrosome becomes flattened, but I cannot determine the manner in which a division takes place. The outer centrosome diminishes in size. Its central astral rays shorten, fig. 19, and ultimately disappear as the apex of the spindle is protruded. The lateral rays are obliterated progressively until the point of the spindle stands clear. The first appearance of the protrusion of the polar body is a tiny elevation into which the end of the spindle is directed. Later, when the spindle has risen to the height of the equatorial plane, there is seen a depression on the surface of the egg where the constriction takes place, and in which afterwards the polar body lies. The rise of the spindle and its protrusion are very difficult to explain. It remains approximately of the same length throughout, and I do not see any special development of the central aster over the polar one. Wilson (1900) finds evidence in the protrusion of the spindle in favour of Dünér's (1895) theory that the divergence of the poles of the spindle in mitosis is due to the progressive elongation of the central spindle. In Echinoderm ova, neither before nor after fertilisation, is there a central spindle spun out between the centrosomes, but it is probable, according to Wilson, that the difference is only a secondary one, and that the spindle consists in part of continuous fibres, and the waviness of the spindle-fibres in the metakinesis would speak for the pushing hypothesis. In any event, I cannot see how any hypothesis founded on mechanical principles, such as illustrated in Heidenhain's model, can explain the peculiar circumstances of the polar mitosis.

Fig. 23 shows the earliest phase of the second division which I have had the opportunity of observing. The asters are already separate, and a bunch of fibres from each is projected towards the chromosomes, which are immediately drawn into the equator of the spindle. Thus no resting stage intervenes between the two divisions. The whole figure is still surrounded by the remains of the reticular or kinoplasmic zone. The spindle when fully formed is slighter than the first polar spindle. The central centrosome and aster progressively increase in size until the condition is found as in fig. 30. The astral rays are thick and fairly straight and widely spreading. The behaviour of the outer centrosome and the manner of protrusion of the polar body is exactly as I have described for the first polar body (figs. 27—29).

Fig. 33 shows the condition of the nucleus long ago described by Hertwig after the extrusion of the second polar body. The first stage in the reconstitution of the nucleus is the formation of several small vesicles, which run together to form a single vesicle which is the mature nucleus. The description given of the process in the living egg is, that several small vesicles appear approximately in the middle of the radiations remaining in the egg. In the sections this is clearly seen not to be the case, but the vesicles surround the centrosome, and the astral rays are broken up into bundles passing out between them. Later these all disappear, and a single vesicle is left without any trace of centrosome or radiation in its neighbourhood.

Fig. 34 shows an interesting abnormality of the second polar body. It is here very distinctly a small cell, and precisely the same phenomena are seen in the reconstruction of the nucleus as in the egg.

I must now refer to a series of figures which accompany the constriction of the spindle in both maturation divisions. Associated with the disappearance of the spindle is formed the body called by Flemming the "zwischenkorper." This plays a considerable rôle in spermatogenesis, but is figured also in a considerable number of the descriptions of polar-

body extrusion. I have seen it in various forms. In fig. 30 are seen round the constricting spindle a series of points which afterwards, as seen in fig. 23, condense to form a ring round the remains of the spindle. It seems to persist for some time, fig. 33, and then disappears.

A final point still remains to be described. When the matured nucleus retires towards the centre of the egg all remains of the reticular or kinoplasmic zone have disappeared, and the nucleus lies surrounded by the alveolar yolk, while round the periphery of the egg the kinoplasmic girdle has narrowed down into a delicate layer of differentiated protoplasm. In sublimate material this is seen as a distinct layer, in which large microsomes are arranged regularly side by side. In the osmic-acid material the distinction is less sharp, but there is generally a difference in the characters of the surface layer. I think that possibly this layer has to do with the formation of the membrane thrown off when the selected spermatozoon enters the egg, and, as has been said, I refer it to the kinoplasmic zone which is differentiated on the breaking down of the germinal vesicle.

History of the Chromatin.—As has been described the greater part of the nuclear reticulum is rejected, and gives rise probably to the reticular zone round the transformed germinal vesicle. Close to the base of the neck of the invading cytoplasm is found an irregular mass of chromatin, just as Matthews describes for *Asterias*, which is presumably the chromatin destined to form the future chromosomes, figs. 5—7. This condensation of chromatin at one point perhaps corresponds to Moore's (1895) synaptic phase, though only a part, not the whole of the chromatin, as in spermatogenesis, is involved in the condensation. Emerging from this condensed mass are seen in figs. 5 and 6 a series of separate elements as to the number of which I am not certain, but I do not think there are more than at a later stage. The following stages, figs. 8 and 9, involve the collection of this mass of chromatin elements into the central plate before described.

Often one sees the chromatin collected to one side of this plate; sometimes the separate elements are widely scattered; in many instances, as in fig. 12, there are chain-like clusters, which suggest that a thread is being broken up into segments, and in practically every ovum at this stage one sees compound masses which are breaking down into the separate elements which enter the equatorial plate of the spindle. Hartmann, as already mentioned, has quite recently described the chromosomes as arising directly from the nucleolus. They arise as isolated rods, clumps, or threads having the chromatin particles arranged in series in them. The nature of my material makes it impossible for me either to deny or affirm the direct origin of the chromosomes from the nucleolus, but the appearances I have described are not otherwise at variance with those described by Hartmann.

I have between fifty and sixty sections of this stage, and the relatively large number indicate that the prophase is protracted. From the very first these always present the same form. Fig. 14 shows a fragment of the thread composed of spheres, united by a less deeply staining substance. When separation is complete sixteen tetradal chromosomes of nearly uniform appearance are found. When seen from the side they have a dumb-bell shape, when seen en face they are obviously the tetradal groups of authors. I have counted the chromosomes at the various stages again and again, and have always reached the number fifteen or sixteen. Fifteen is an improbable number, and I feel sure that the proper figure is sixteen. I have never succeeded in making the number eighteen, which would be double the number (nine) found by Boveri (1890) in *Echinus microtuberculatus*. R. Hertwig (1896) made the number of chromosomes emerging from the germ nucleus, in his experiments on the development of unfertilised sea urchin eggs, sixteen or eighteen, which would agree with my results. Field (1893) in Echinoderm spermatogenesis counted twenty-six to thirty-two chromosomes in the spermatogonia, sixteen to

eighteen in the spermatocytes, and eight or nine in the spermatids. He confesses to great uncertainty in regard to these figures on account of the minuteness of the chromosomes, and the last figure is quite out of harmony both with R. Hertwig's counts and my own.

Careful analysis of this tetrad body shows that it is composed of two short stout rods placed side by side (figs. 8—10, 12, and 23). The ends have the form of little spheres, and looking back to fig. 14 one may conclude that they correspond to the spheres seen in that thread united in pairs, but there is no transverse cleavage of the thread between the four spheres. A complete tetrad, consisting of four independent round bodies as figured for *Ascaris*, or the mole-cricket, does not occur in *Echinus*. Further, one cannot regard the two rods as separate and independent at this stage; they are bound together closely, and the figure is really a compound chromosome.

According to the above interpretation the tetrads thus arise by a single longitudinal split of an original thread or threads. At no time are there any ring or other irregular figures, as described in so many other cases. The possibility is not excluded, that the groups might result from conjugation of the dyadal bodies in pairs, as described by Wilcox (1895) in the grasshopper, and Calkins (1895) in the earthworm. In two instances only out of a large number of prophase stages have I seen a figure other than those described. In one section, just before the spindle is formed (fig. 15) there is a double comma form, which appears in all other sections at a later stage.

As the compound chromosomes are gathered into the equatorial plate they lie irregularly, and in the metakinesis they do not seem to be resolved simultaneously, for in all my sections of this stage, about sixty in number, figures in different phases are seen, and as the chromosomes lie throughout the whole equatorial plate, and not only round the periphery of the spindle, various irregular bodies are seen which are portions only of whole chromosomes. The

relatively large number of sections obtained in this stage indicates that it is of long duration.

The varied figures drawn in figs. 18 and 21 are capable of only one satisfactory explanation, keeping in view that the end result is always the same. The little rods come to be placed radially on the spindle. Their central ends move apart to form a **T**-shaped figure. The cross-piece of the **T** representing the separating limbs opening out on the spindle, the stem of the **T** the outward directed, and still united portions of the chromosomes. As separation proceeds the stem of the **T** is pulled down until the figure is like two commas placed end to end. It is obvious that this evolution will open out the chromosome along the plane of the original longitudinal split from within outwards, as is seen in a series of drawings (fig. 21) of the chromosomes in profile view, but when observed en face (same figure) it is equally clear that a second longitudinal split has simultaneously been effected along a new plane, from without inwards, giving the double **V**-shaped figures represented in figs. 18, 19 and 21.

If we describe the appearance in terms of the minute terminal spheres of each rod, we see that the spheres come to lie in a row exactly as Wheeler (1897) describes in *Myzostoma glabrum*. The equatorial bodies then divide (figs. 16—18 and 21), but the terminal spheres of each rod remain undivided, and are drawn away from the equatorial spheres, so that the whole chromosome is lengthened out very greatly, and the apical spheres are carried far away from the equatorial, delicate, less deeply staining threads uniting them together. The equatorial spheres, after remaining long in contact in the equator, then part, and give rise to **V**-shaped figures with a single apical and two equatorial spheres, one at the end of each limb. These figures then shorten up by the contraction of the elongated thread, and in the final anaphase condense into short stumpy masses (figs. 19 and 21).

These, when analysed, show that the apical sphere has also divided, and we have produced small tetradal bodies exactly like those in the prophases of the division, but of smaller

size. In reality, just like the earlier bodies, they are short, somewhat curved rods, with dilated extremities placed side by side. Those at the outer pole pass into the first polar body, and those remaining in the egg persist, enlarge somewhat, and pass otherwise unchanged into the second polar spindle. Sometimes during the metakinesis the second longitudinal split is not so evident, and then long drawn out threads are seen, the double nature of which is difficult to make out. Ultimately, however, the two halves separate in the anaphase exactly as in other cases. Exactly similar figures have been described by quite a number of observers in other forms, for instance, and especially distinctly, by Klinckowström (1897), Francotte (1897), Van der Stricht (1898), Griffin (1899), Gathy (1900), but, as I shall describe in the sequel, their interpretation has been different, and leads to very different theoretical conclusions. The transition between the first polar and the second polar spindle is very rapid, so that the number of sections found in this stage is relatively few.

The little compound chromosomes are drawn into the equatorial plate of the second spindle (figs. 23 and 24), and there different appearances are seen, according to the plane of the section. In fig. 24 we have apparently little tetrads, which are really the lobed ends of the small, slightly curved chromosomes. In fig. 25 again the rods are seen lying back to back. These rods I have every reason to believe, from the various figures I have drawn, open out just as in the first spindle, only there is no second longitudinal split, and therefore the division is homotypical. A single preparation rather suggests that the rods may sometimes be simply separated along the plane of cleavage. It may well be that both methods are adopted, according to whether the body lies radially or tangentially to the spindle. The result is the same; the separation is effected in the plane of cleavage established in the anaphase of the first division.

Similar figures in the second division have been described by the authors above mentioned, and in other instances,

also, the short, slightly curved rods have somewhat the appearance of tetradal groups. When the daughter chromosomes have separated they pass to the poles of the spindle. Those at the external pole pass out with the second polar body, and remain as short, stout, distinctly bilobed bodies in many instances, after the second polar body is cut off (fig. 31). Those remaining in the ovum, however, at once begin to lengthen, and in the telophase are seen (fig. 30) as long, bent rods. These are gathered into the series of vesicles already described. Within each of these vesicles are seen elongated, curved rods, and round the walls there are tiny particles of chromatin, forming an incomplete membrane (fig. 33). Later, when the vesicles are fused, the nucleus is seen to be bounded nearly all round by semicircular loops of chromatin, and in the centre the reticulum is becoming restored (fig. 31). At a later stage (fig. 32) the reticulum takes on the form of irregular feathery strands, beset with chromatin granules of varying size, accumulated here and there to form irregular net-knots of chromatin. All trace of the separate chromosomes is absolutely lost in this network.

The phenomena attending fertilisation and cleavage are so well known that I do not intend to enter on that subject, but I wish to refer to the behaviour of the chromatin threads in the metaphase of the cleavage division. The primary rods segment into about thirty-two chromosomes. I have counted them in cross sections of the spindle a good many times, and generally reach that figure, which would make my count of the chromosomes in the maturation stages fall in exactly with the general law.

Each chromosome when divided forms first a V-shaped figure. This mounts on the spindle so that a loop is formed with its apex directed outwards, and the ends of this loop are drawn out to the poles of the spindle, the threads lengthening as they go. Finally, the daughter chromosomes separate by the breaking apart of the thread at the point which corresponded to the apex of the loop.

This is exactly the manner in which I have described the short, stout chromosomes of both maturation divisions as opening out on the spindle. The difference between the two types consists only in the stoutness of the chromatin rods in the polar mitoses, the occurrence of a second longitudinal split in the first division, and consequently the absence of the usual longitudinal cleavage in the second division.

Summary of Results (Text-figs. 2 and 3, pp. 213, 214).—The chromatin thread or threads, derived only from a portion of the mass of chromatin in the germinal vesicle, are found split longitudinally and segmented into sixteen bodies—half the number of the chromatin rods in the nuclei of the cleavage divisions. These bodies consist of two short rods placed side by side, and each rod is composed of two spheres united by a less deeply stained portion of the thread. The two rods are intimately associated so as to form a tetrad-like mass, and the whole figure is to be considered a compound chromosome.

After a relatively long prophase each of these is resolved in the first polar metaphase, in such a manner that while the body is opened up along the original cleavage plane, another longitudinal cleft is effected, which is completed in the anaphase, and the final result is another compound chromosome exactly like the original from which it sprang except in size. Each of the sixteen double rods which remain in the ovum after the extrusion of the first polar body is resolved in the second polar spindle into its two elements without further cleavage taking place.

In the telophase of the second division the elements which remain in the ovum after the extrusion of the second polar body elongate into rods which become bent on themselves, while those in the second polar body remain condensed as small bilobed rods.

The maturation phases differ from the ordinary cleavage mitoses in respect of (*a*) the thickening and condensation of the chromatin rods, (*b*) the second longitudinal splitting which occurs in the first metakinesis, and (*c*) the absence of

longitudinal cleavage in the second metakinesis. The second mitosis thus merely distributes the granddaughter chromosomes formed by the second longitudinal splitting in the first mitosis.

There is thus no "reducing division." The only reduction which occurs is effected in the germinal vesicle, and the chromatin destined to form the chromosomes of the polar divisions is diminished in bulk merely.

Critical Analysis of Results and Comparison with those of other Observers.—In describing the achromatic structures I have sufficiently indicated how the appearances I have described in my material are to be compared with those described by other observers. With regard to the chromatin elements I may now give a further analysis.

Glancing over the whole field of research on the subject the first thing that strikes an observer is the remarkable unity of the process, even in detail, over a very large range of forms. The figures represented for the great majority of both the higher plants and the Metazoa show resemblances so close that one cannot imagine they are produced in one way in one form and in another way in another form. Interpretation and theoretical conclusions may differ, the process is identical throughout.

It has been insisted that the solution of the problem of reduction lies in the determination of the origin of the tetrads, but as these in typical form occur in a relatively small number of cases, it seems that the solution rather lies in a closer analysis of the heterotypical division, such as has lately been done for plants by Strasburger.

Heterotypical division was first described by Flemming, in 1887, as a form of mitosis occurring in the spermatocytes of the salamander, and in all cases in which tetrads are not formed a heterotypical division in some sort ushers in the first maturation division with its reduced number of chromosomes, and this is true of plants as well as animals. The distinctive features of this division as originally stated are:

1. The spireme stage is not so compact as in other kinds

of cells. 2. The sister threads round which the segments split are fused by their ends up to the metakinesis. 3. The monaster stage is short lived, and shows a radial arrangement only indistinctly on account of the twisted position of the threads. 4. The end stage of the metakinesis is very prolonged, and has a very special character, in consequence of the fusion of the ends of the threads. 5. A temporary and not understood second longitudinal cleavage of the threads appears in the anaphase. The outstanding feature of the heterotype was considered at first as being the incomplete separation of the two halves of the longitudinally split rods resulting in the formation of ring chromosomes, but the figures may assume very various forms according as the loop is bent, or drawn out so as to obliterate the hollow of it. Again, the rings or their derivatives may be attached to the spindle in different fashion, so that in their resolution different irregular figures emerge. This is shown in the series of diagrams given in the paper of Farmer and Moore, who first clearly pointed out the essential resemblance of the heterotype in plants and animals. The feature described by Flemming, namely, the second longitudinal cleavage found in the anaphase, seems until recently to have had very little significance attributed to it.

The simplest idea of heterotypical division is that the two halves of the ring-shaped chromosomes are drawn out into U- or V-shaped daughter loops. This simple explanation will not explain many of the figures observed. Farmer (1895), in a study of the phenomena in the lilies, described a double cleavage taking place simultaneously in different planes as the compound chromosome is resolved into its daughter elements. In 1896, along with Moore, he gave an explanation of the phases, which only involved one split, the second being merely apparent. The idea elaborated was that the elliptical ring was bent on itself, applied to the spindle at its apex, and then drawn out to the poles from the point of bending. The original ends were ultimately broken across at the equator. Moore, in his work on

Elasmobranch spermatogenesis, adopted this explanation of his figures. Gregoire (1899), describing the stages in lilies, and Strasburger in his recent work, from a careful examination of the prophase in a large number of plant forms, absolutely decide against the idea of the bending up of the ring. In *Echinus*, where no ring is seen at any time, the explanation is easier and more direct, and the facts decide conclusively against such an interpretation. Strasburger, in addition to examining a large series of cases, reviews the results of other observers, and comes to the general result that all the processes can be referred to one type, namely, (1) As the result of the primary longitudinal cleavage of the chromatin thread, two rods, wavy or curved, are formed. These straighten and ultimately shorten down into stout rods. In shortening, various adhesions and twistings may take place, so as to form rings or twisted threads. (2) According to the position assumed by these various figures on the spindle, the character of the resulting metaphase figures depends. (*a*) If the chromosomes are placed radially in the form of two rods side by side, they are drawn apart in the plane of the first cleavage, and at the same time a second slit is effected from the free end inwards. The result is the formation of V-shaped daughter chromosomes, which in the anaphase break apart at the apex to complete the second longitudinal cleft. (*b*) If placed tangentially the result depends on the point of attachment of the "zugfasern," but invariably as the limbs are drawn apart, a second longitudinal cleavage reveals itself, and two daughter V's are formed. The first type (*a*) is exactly what I have described in *Echinus*; the second form (*b*) is exactly that described in amphibians.

Flemming (in 1887), and Meves (1896) in *Salamander*, McGregor (1899) in *Amphiuma*, Kingsbury (1899) in *Desmognathus*, give us conclusions in the main identical. All describe and figure a second longitudinal cleavage of the chromosomes in the dyaster stage, and this cleavage is preparatory to the second division. The nucleus is

partly reconstructed between the divisions, and the longitudinal cleft is lost sight of, to reappear in the second division either by re-establishment of the old or by a new longitudinal splitting. Kingsbury was able to trace the longitudinal cleft directly into the second division, owing to the fact that the nucleus is not so far reconstructed. More recently (July, 1900) Janssens described the phases in Triton, and took the further step of interpreting the process in exactly the same terms as Gregoire in lilies. Flemming (1887), in his first paper, described tetrads, but regarded them as abnormal. Vom Rath (1893) redescribed these bodies as normal appearances, but his results were not maintained by Meves (1896), who failed to find the least evidence of tetrads in amphibian spermatogenesis, though he, in a short paper, described tetradal figures as an abnormality in the early oocytes.

Amphibian oogenesis has been attacked by Fick (1893) and Born (1894), and Carnoy and Le Brun (1899). Practically identical figures are given by all three, but the later authors give much more complete details, and offer a new interpretation. They describe the chromosomes as condensing after some intermediate phases into short rods. These are complex structures formed by the fusion of a considerable number of separate elements. These short rods, or rather blocks, place themselves in the equatorial plane of the spindle in a circle round its periphery, and orient themselves so as to be placed with their thicker and larger ends on the spindle, the other end being directed outwards. Once installed in this position, the chromosomes go through varied movements, during which they submit to a double longitudinal splitting. The one is effected in the equatorial plane, the other in the axis of the spindle and perpendicular to the first. The equatorial division shows itself first and begins in the large part attached to the spindle, rising insensibly into the stalk. The second occurs later, and begins at the summit of the stalk, descending by degrees till a kind of tetrad is formed. Further complicated changes are described, which result in

double V's originating in the wings of the "equatorial crown." These are separated and carried to the poles. A partial reconstruction takes place in the anaphase, but V's again appear in the telophase, and the double V's remaining in the ovum are separated from one another in the second polar spindle. The whole description shows a very complicated process, and exactly what I have found in *Echinus* in a much simpler form, because as there are no V's or twisted threads to complicate the picture, I may say that only the initial stages described by Carnoy and Le Brun are found in *Echinus*.

This is the only positive evidence of the occurrence of a simultaneous double split of the compound chromosome of the heterotypical division in animals. It will be seen that my interpretation agrees in the main with that of Carnoy and Le Brun, and Janssens; and further, that the same idea has enabled Strasburger to reduce the heterotype in the higher plants to one common plan.

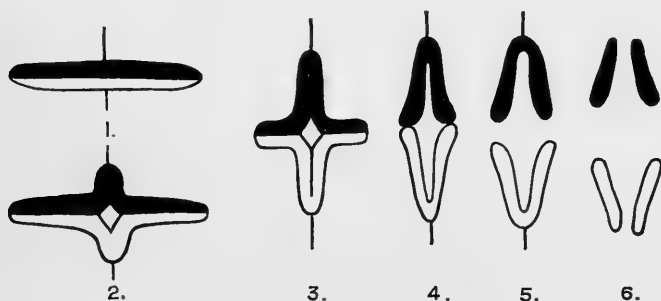
Now *Echinus* falls exactly into line in every essential respect with another considerable series of cases recently described.

1. *Prostheecræus*, Klinckowström, 1897.
2. Various *Polyclads*, Francotte, 1897.
3. *Thysanozoon*, Van der Stricht, 1898.
4. *Thalassema*, Griffin, 1899.
5. *Zirphæa*, Griffin, 1899.
6. *Tubifex* and *Clepsine*, Gathy, 1900.

In every one of these the figures belong to the same type, except that Van der Stricht and Griffin describe rings in the prophases. The last observer has not given details, because the chromosomes were too minute for analysis.

The first four authors all explain their results according to the diagram given below (Text-fig. 1). The double rods resulting from the compression of the ring are placed with the longitudinal cleft in the plane of the equator of the spindle, and are drawn apart by their middle points to form U-shaped or V-shaped figures, and the breaking

apart of the U's or V's at the apex in the anaphase is held to be a transverse division of the original long chromosomes. Griffin alone says that the possibility of a second longitudinal cleavage is not absolutely excluded, but as the T-shaped figure is rare, he held to the other explanation. It is obvious that it is extremely unlikely that such exactly similar appearances should arise in different ways. I believe that the demonstration I have given of the nature of the process as it is seen in *Echinus* might, if applied to them, reconcile all these instances with what is known to occur in *Amphibia* and the higher plants. A certain part of the contradiction in results would thus be removed,



TEXT-FIG. 1.—Scheme showing the successive stages in the resolution of the chromosomes in the "heterotypical divisions" according to an interpretation which makes the apical break of the V a transverse cleavage. (After Wilson.)

and instead of these cases being held to prove a reducing division in Weissmann's sense, they would, as does *Echinus*, disprove it.

The figures given by Linville (1899) for certain pulmonate Gastropods are very similar to those described in this group, but the origin of the figures is not completely worked out. He decided for a longitudinal division in the first division, and the elements are doubled in the anaphase, while these again are distributed in the second division.

On the other hand, in *Helix pomatia*, Bolles Lee (1897) describes appearances which lead him to conclusions different from most other observers. He finds transverse divisions in

both mitoses, but no reduction in the number of chromosomes. He holds that there is both quantitative and qualitative reduction. His figures have a strong family resemblance to those in *Echinus*, but the chromosomes are very lumpy and solid, and do not show the compound character of their prototypes which I have described. Bonin and Collin, in a recent paper on the "Mitoses in the Spermatogenesis of *Geophilus linearis* (Koch)," also interpret the appearances as due to two successive transverse divisions.

This is a very good example of the extraordinary variety in the manner of interpretation of closely similar appearances, which is evidence of the great difficulty of reaching any degree of certainty in cases where the chromosomes are small and numerous.

I come now to another series of cases in which the so-called tetrads play a large part. A figure consisting of four separate spherical bodies is very rare, occurring only in *Ascaris* and the *Insecta*, and can be explained in two different ways. First, in *Ascaris*, it seems, from the researches of Boveri (1897), Hertwig (1890), and Brauer (1893), that the primary chromatin rods split twice longitudinally, preparatory to two rapidly following divisions which succeed one another without a pause. Two groups of four rods are formed, which condense into two tetrads. In the first maturation spindle two of these are linked together as dyads, and pass to the poles of the spindle. The dyads retained in the ovum are resolved into monads in the second maturation division. Whilst there is a mass reduction there is thus no reducing division, no dissimilar distribution of the "ids" of the original spireme thread.

Second, Henking (1891) described in *Pyrrhocoris* tetrad groups which arose in another way, by a single longitudinal and transverse cleavage of the spireme thread, and interpreted the first division as a reducing, the second as an equation division. Vom Rath (1892) followed this account by a description of the process in *Gryllotalpa*, the mole-cricket, in which he figured the halves of the split rods remaining

united to form rings. The chromatin material was then condensed on to four parts of the rings, which broke up to form typical tetrads. These were distributed as dyads in the first polar, and monads in the second polar spindle. Vom Rath held that each of these bodies represented a single chromosome, and that both divisions were "reducing." There is thus not an "equation division," but a dissimilar distribution of the "ids" of the spireme thread. In neither of these cases is the first maturation mitosis of the heterotypical form. Vom Rath's results were partly corroborated, partly modified, by Wilcox (1896) for the spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen*. The difference between the two interpretations is that Wilcox found the tetrad formed by conjugation of dyads, and reduction consisted, therefore, not in the unequal distribution of sister "ids" lying next each other in the spireme thread, but of any "ids" indifferently from any part of the spireme thread. Paulmier (1899), in *Anasa*, described the formation of the tetrads more in the fashion described for the Copepods by Rückert and Haecker, except that there is no spireme stage, and his first maturation division is unequal, owing to the manner in which the tetrad groups are placed on the spindle, separation taking place in the original transverse plane. The second division is an equal division, the separation being effected along the original longitudinal plane of the tetrad. It is to be noticed that his tetrads are not composed of four separate elements, but are compound bodies, the elements of which are condensed into a homogeneous mass. Rückert (1894) and Haecker (1892-3) examined a considerable series of Copepods. They found the earlier stages to differ in the various forms, but the end result was always the same, namely, a condensation of the elements into tetrad groups. The early stages differed according as the split of the primary rod was complete or incomplete at one or both ends, the result being the formation of double rods, angles, or rings.

Among the Copepods the case of *Cyclops brevicornis* (Haecker, 1895) requires special mention. The splitting was

here complete, and double rods were formed, which divided transversely, and then united again by their ends, so that the original tetrad figure was replaced by a pair of rods lying side by side. Rückert and Haecker, in explanation of their results, adopted the "apparent reduction" hypothesis,—that is, the reduction is only apparent in the first division, and is realised in the second, by a suppression of a second longitudinal splitting. It is to be particularly noticed that in *Cyclops* Haecker makes the chromosomes of the second division bivalent.

It is interesting to note that, except in insects, the type, which Haecker calls the plant type, has claimed most of recently described cases. In this type, as before explained, the typical tetrad formation is absent, and Haecker homologises the rings described in the prophases with the tetrad groups by making them equivalent to the four elements of these bodies. Griffin has attempted to establish the same analogy by imagining his cross-figures as derived from a crushed ring, the four limbs of which represent the four bodies in the tetrad.

Echinus will not fall into any of Haecker's types. The special value of the observations in *Echinus* seems to me that the heterotypical division is present without previous ring formation on the one hand, and on the other distinct tetrads are formed which certainly submit to a second longitudinal division.

I shall now endeavour to explain my results in terms of the tetrad, but I must first of all refer to Boveri's and to Wilson's figures of the second polar spindle in *Echinus microtuberculatus* and *Toxopneustes*. They both show obvious dyads in the equatorial plate, exactly as seen in *Ascaris* and *Grylotalpa*, and this was the interpretation I was at first inclined to give to the appearances, until I convinced myself of the compound nature of the bodies, which at once transferred *Echinus* from the group represented by the insects to that represented by the more recently described case of the Turbellarians, that is to Haecker's plant type, though, as I

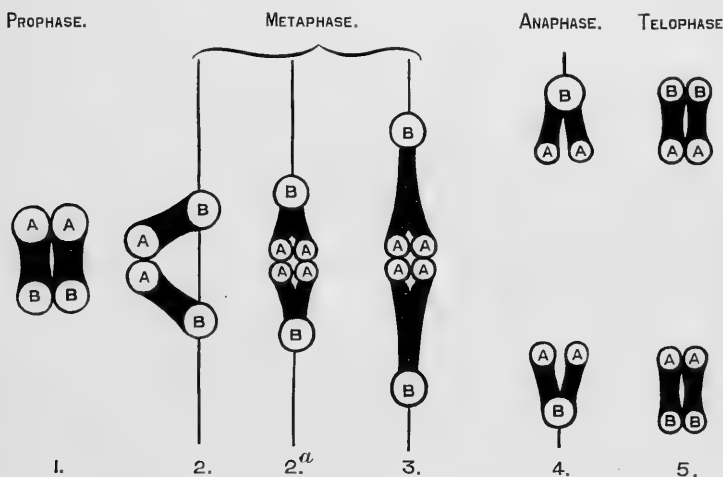
have said above, it differs from that type in certain important particulars, and agrees closely with the Cyclops type.

Each half of the compound chromosome or tetrad is a short rod, showing at its ends small spherical bodies. If these spheres are to be interpreted as separate elements of the tetrad, there being absolutely no trace of a second longitudinal

division, I cannot represent the figure as in *Ascaris* $\frac{a}{a} \mid \frac{a}{a}$. If one adopted Haecker's idea of a suppression of the last transverse segmentation of the spireme thread, the figure could

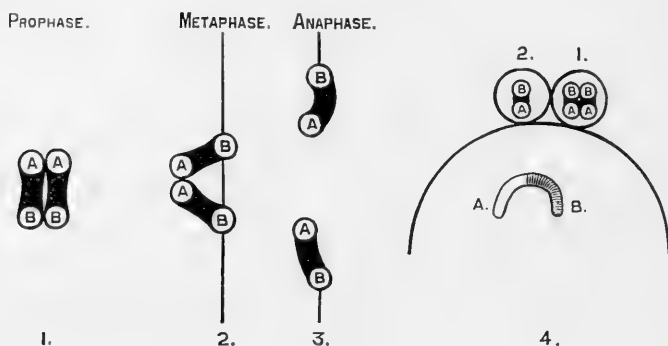
with perfect propriety be represented $\frac{a}{b} \mid \frac{a}{b}$, assuming each

sphere to be the equivalent of a single chromosome. Following up this formula through the first and second divisions, it would work out as follows:



TEXT-FIG. 2.—Scheme of first maturation division.

1. Double-rod prophase figure or tetrad; first longitudinal split.
2. Double-rod figure placed radially on spindle; opening out of daughter chromosomes in plane of first cleavage. Profile view.
- 2a. Same in face view showing beginning of second longitudinal split.
3. Elongation of chromosomes.
4. Separation of daughter chromosomes. Each is much contracted, and the second longitudinal split has further extended, so as to give rise to a V.
5. Completion of second longitudinal split, converting the V's into double-rod figures, which are the granddaughter chromosomes.



TEXT-FIG. 3.—Scheme of second maturation division.

1. Double-rod chromosome = granddaughter chromosomes produced in anaphase of first division.
2. Opening out of longitudinal split established in first division.
3. Separation of granddaughter chromosomes.
4. Large half-circle represents ovum; within it a granddaughter chromosome elongated into a curved rod. Smaller circles represent polar bodies; in 1 a double-rod chromosome resulting from the second longitudinal split of the daughter chromosomes of the first division, in 2 a single bilobed body, the granddaughter chromosome.

I have given reasons for my belief that the sphere-like portions of the rod can be identified through the heterotypical division, and that each submits to a division in the

process; and if we presume to call the first figure $\begin{array}{c} a \\ | \\ b \end{array}$ it must

necessarily follow that the elongated loops in the telophase of the second polar spindle, and the bilobed rod in the second

polar body, must also be labelled $\begin{array}{c} a \\ | \\ b \end{array}$, and the final result is that

the apparent reduction is not confined to the first division, but is maintained throughout,—in other words, that the chromosomes are coupled in pairs, and go through their evolutions as linked chromosomes. Now, returning to the case of *Cyclops brevicornis*, Haecker regards each half of the double rod of the first metaphase as the result of a fusion of two elements end to end—so that each is bivalent, though they go through their evolutions as if they were univalent rods. Thus, if each half of my tetrad figure were bivalent, our results would up to

this point agree. In the second polar metaphase I find double-rod figures, which are the granddaughter chromosomes lying side by side—each being bivalent like the daughter chromosome from which it sprung. Haecker, in the second metaphase, finds half the number of elements seen in the anaphase of the first spindle, and he accounts for his pseudo-tetrad figures by supposing that the previous anaphase figures become linked together. According to his account, there is no second longitudinal splitting apparent, and therefore the elements are daughter, not granddaughter chromosomes joined together. Each of the daughter chromosomes is bivalent, so that when united, a complicated redistribution of the elements is brought about according to the formula—



and separation being effected in the plane of the last transverse segmentation of the spireme thread, there is a true reducing division. Haecker suggests several possibilities in explanation of the figures, that just given being his choice; it does not seem very convincing to me, and the figures lack the inevitable sequence which is apparent in *Echinus*.¹

Returning to my own results, we can only on theoretical grounds assume that each sphere represents a separate chromosome; but the idea certainly provides a plausible explanation, though, of course, such an interpretation deprives the process of any significance such as Weissmann and

¹ While this paper has been passing through the press Lerat has published in the 'Anatomischer Anzeiger' a preliminary note on the first maturation division in *Cyclops strenuus*. He does not adopt the explanations of Rückert and Haecker, but brings the first mitosis into Strasburger's scheme of the heterotypical division, in which the longitudinal division of the daughter chromosomes is a fundamental character.

others have attributed to it. If we look upon the tetrad as a single chromosome longitudinally divided, then we cannot get beyond the statement that during maturation the chromatin substance, which is retained to form the chromosomes, condenses into masses, which are half the number of the segments characteristic of the cleavage nuclei, and that these masses adopt a special form in the prophase of the first division, preparatory to the occurrence of a double longitudinal splitting.

It would be tedious and unprofitable to attempt a complete recapitulation of all the cases described. In many forms, owing to the difficulty of obtaining an absolutely complete series of stages, the evidence is incomplete, while in others the minuteness of the chromosomes is a barrier to finer analysis. I must, however, refer briefly to the facts as represented by the botanists regarding the heterotypical division.

In the earlier days of investigation into the mitosis occurring in the pollen mother-cells of higher plants, Strasburger (1888) and Guignard (1891) described a longitudinal splitting at the beginning of each division, and in regard to reduction of the chromosomes they did not find that the pollen mitoses differed from the process in vegetative cells.

Belajeff (1894) was the first to point out that the V-shaped figures of the heterotype were not due to the rods or rings being curved progressively in their ascent to the poles of the spindle.

Farmer (1895), as I have already said, described an apparent double longitudinal cleavagesimultaneouslyprogressing, but in his paper in conjunction with Moore he elaborated the idea that the double rod of the prophase was produced by bending of the ring on itself and the fusion of the two halves. In the metaphase the rods were separated along the plane of fusion, so that only a single longitudinal cleavage was involved, and the separating elements were the original daughter chromosomes. He held that there was a longitudinal splitting of the chromosomes in the second division.

Strasburger (1895) gave an explanation involving two

longitudinal cleavages, the second split completing itself in the anaphase preparatory to the second division.

Dixon (1895) gave a somewhat different explanation, involving a longitudinal split taking place for the first time in the metaphase.

Miss Sargent (1895) described two longitudinal splits in the primary chromatin thread, but adopted an idea of the heterotype, which was essentially similar to that of Farmer's second interpretation.

Ishikawa (1897) in *Allium*, and Calkins (1897) in *Pteris*, described for the first time tetrads in plants. According to the description of the former observer, these tetrads were resolved in the heterotypical division in such a fashion that when the daughter chromosomes broke at their apex a transverse cleavage was completed.

Strasburger and Mottier (1897), under the influence of the idea of the bending of the ring and its subsequent resolution along the same plane, admitted the possibility that the separation of the **V** figures occurring in the prophases of the second division was a transverse splitting, but a few months later these authors thought they had discovered a longitudinal division during the prophase of the second division.

Belajeff (1898) pronounced for a transverse division in Weissmann's sense, but Guignard (1899), for *Najas major*, returned to the interpretation proposed by Strasburger in 1895 for the lilies.

Grégoire (1899) made a re-examination of the phenomena in the Liliaceæ, and concluded for a double longitudinal cleavage, the daughter V's in the first division being separated without further cleavage in the second.

The difficult point in the heterotype in higher plants—a single longitudinal split being admitted in the prophases—is to account for the **V**-shaped forms and their varieties. Strasburger, after changing his ground several times, returns in his last pronouncement (1900) to his ideas of 1895 with some modifications. He finds, as I have already said, the **V** figures arise in two ways, according to the position assumed

by the prophase figures on the spindle, but in every case a second longitudinal cleavage takes place.

An examination of his plates shows how remarkably closely the figures I have drawn for *Echinus* resemble, even in their details, those he has figured for the plant forms, and the general statement of his conclusion is as remarkably in conformity with my own. He says (p. 81), "The special peculiarity of the first nuclear division of the spore and pollen mother cells, which follows the numerical reduction of the chromosomes, consists in this, that the daughter chromosomes, which arise by a longitudinal splitting of the mother chromosomes, are inclined to a premature separation, and that they directly suffer a second longitudinal cleavage.

"The second nuclear division, which follows on the reduction of the chromosomes, has only the mission of distributing to the granddaughter cells the granddaughter chromosomes already produced in the first division.

"The two divisions differ from ordinary mitotic division only in the double longitudinal splitting in the first mitosis, and the condition thus created for the second division." Again (p. 99), "The pith of the heterotypical division lies in the two longitudinal clefts, not in the form of the chromosomes."

"The cause of the two cleavages of the chromosomes so rapidly following one another, which again conditions the rapid sequence of the two nuclear divisions, must lie in the process of reduction which precedes the maturation division."

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EXPLANATION OF PLATES 10-12,

Illustrating Dr. Bryce's paper on "Maturation of the Ovum in *Echinus esculentus*."

All the figures were drawn by aid of the camera lucida with Zeiss 2 mm. apochromatic objective of 1.40 mm. aperture (homogeneous immersion), and 6, 8, or 12 compensating ocular. The magnification, tested by the stage micrometer, of Figs. 1 to 6 is 850, of the remainder 1200, except Fig. 20, which is magnified 1500 times. The drawings represent as realistically as possible what can be seen in the individual section, and, except in Fig. 18, no attempt has been made to make the drawing diagrammatic by incorporating data from two or more sections of the same ovum. A series of photo-micrographs of the stages, kindly taken for me by Dr. J. H. Teacher, was of considerable assistance for comparison with, and verification of, the drawings.

PLATE 10.

FIG. 1.—Early oocyte.

FIG. 2.—A stage in the growth of the germinal vesicle, showing also the appearance of the cytoplasm when the preparation is not strongly washed out.

FIG. 3.—Another phase in the growth of the germinal vesicle, showing also the appearance of the cytoplasm in a preparation from which the iron hæmatoxylin has been strongly washed out.

FIG. 4.—Section through invagination into germinal vesicle.

FIG. 5.—Disappearance of nuclear membrane; rejection of greater part of chromatin network; isolated mass of chromatin from which chromosomes are derived.

FIG. 6.—Later stage, in which two asters have appeared.

FIGS. 7 *a* and 7 *b*.—Two adjoining sections through the same germinal vesicle, showing the mass of chromatin from which chromosomes will be formed.

FIG. 8.—The two definitive asters; chromosomes in form of bilobed bodies or tetrads.

FIG. 9.—Another section, showing the chromosomes within a central plate, surrounded by a crown of radiations fading imperceptibly into the cytoplasm; one aster in view, a second lay in the adjoining section. Sometimes a third aster is seen at this stage.

PLATE 11.

FIGS. 10—14 represent the different forms assumed in various sections by the chromosomes before the formation of the first spindle. The dotted lines indicate the outline of the central plate.

FIG. 15.—Asters now in radial position; early phase of first division spindle.

FIGS. 16, 17.—Two phases of metaphase of first division.

FIG. 18.—Same stage semi-diagrammatic. The various metaphase figures in two adjoining sections of the same ovum are each carefully drawn, but represented in the same plane.

FIG. 19.—Anaphase of first maturation division.

FIG. 20.—Slightly oblique section of constricted spindle in formation of first polar body.

FIG. 21.—Various chromosomal figures seen during progress of first division, in profile and face views.

FIG. 22.—The chromosomes retained in the ovum after the extension of the first polar body, as seen in an oblique section of central aster.

FIG. 23.—First polar body and centrosomes of second division before formation of spindle.

FIGS. 24—26.—First polar body and second polar spindle in metaphase, showing the chromosomes in various aspects.

PLATE 12.

FIGS. 27 and 28.—Two stages in the anaphase of second polar division.

FIG. 29.—Constriction of spindle in formation of second polar body.

FIG. 30.—First polar body and second polar spindle in telophase. Section is oblique, so that second polar body is not seen. The point of constriction of the spindle shows one form in which the mid-body occurs.

FIG. 31.—The two polar bodies and the reconstituted nucleus.

FIG. 32.—The completely re-formed and matured nucleus, which has retired to near the centre of the egg.

FIG. 33.—An early phase in the reconstruction of the nucleus; a number of vesicles are seen which run together to form the single vesicle seen in Fig. 31. The remains of the spindle and mid-body are also seen.

FIG. 34.—Abnormality of second polar body. It is a small cell in which the nucleus is being reconstructed exactly as in the ovum.

Studies on the Arachnid Entosternite.

By

R. I. Pocock.

With Plates 13 and 14.

THE investigations here recorded were set on foot in the first instance with the purpose of settling certain contradictions as to matters of fact in the extant descriptions and published figures of the entosternites of various Arachnids, which a preliminary dip into the literature revealed. It was necessary to ascertain whether these discrepancies were attributable to a natural variability in the organ, to specific or generic differences between the species dissected, or to errors of observation on the part of the dissectors. In some cases, too, there was an entire lack of agreement on the part of observers in the interpretation of the facts recorded; and the suggested homologies between the constituent parts of the entosternites of various species did not, on a priori grounds, appear to be in all cases satisfactory. I was anxious, moreover, to test the respective claims to recognition of the two theories of the origin of the entosternite that have been put forward.

I have made no examination of this organ in the Palpigradi, Pseudoscorpiones, Podogona, Opiliones, or Acari, and have nothing to add to what has already been said about the entosternite of these orders.

The contents of this essay, which deals exclusively with the remaining existing orders, may be tabulated as follows :

I. The structure of the entosternite in the Xiphosuræ, Scorpiones, Pedipalpi, Araneæ, and Solifugæ.

1. The entosternite of the Xiphosuræ, p. 226.
2. " " " " Scorpiones, p. 227.
3. " " " " Pedipalpi, p. 231.
4. " " " " Araneæ, p. 233.
5. The "so-called" entosternite of the Solifugæ,
p. 237.

II. The comparative morphology of the entosternite, p. 239.

III. Theories of the origin of the entosternite, p. 247.

I. STRUCTURE OF THE ENTOSTERNITE IN THE XIPHOSURÆ, SCORPIONES, PEDIPALPI, ARANEÆ, AND SOLIFUGÆ.

1. The Entosternite of the Xiphosuræ.

The form and structure of the entosternite in the American *Limulus* is well known, thanks to the figures and descriptions of it published by Ray Lankester (5, 6) and Benham (2).

It is a longitudinally oblong plate, with a pair of stout anterior bars, or cornua, forming the pharyngeal notch, two pairs of long and slender apophyses behind the anterior bars, diverging nearly at right angles from the main body of the plate, and a stout but short apophysis springing transversely from its postero-lateral angle on each side. There is also an irregular-shaped posterior median process, as well as a pair of short apophyses projecting subvertically beneath the latter. In the Moluccan species *T. gigas* (= *moluccanus*), as was shown by Van der Hoeven, there is only a single long apophysis projecting from the lateral border in front. This represents the anterior of the two that are found in this position in *X. polyphemus*. This peculiarity obtains also, I find, in the other Asiatic species, *Tachyples tridentatus* (= *longispina*) and *Carcinoscorpius rotundicauda*, thus confirming the opinion I have already put forward ('Ann. Mag. Nat. Hist.,' April, 1902) that these

three forms belong to a group distinct from and more specialised than that of *polyphemus*, as is clearly shown by the structure of the genital operculum, etc. Also the pair of posterior ventral apophyses found in *polyphemus* are missing in the two young specimens of *tridentatus* and *rotundicauda* I have examined. In all species of *Limulus* the upper side of the entosternite is furnished laterally behind the middle with a short muscle-bearing excrescence, suggesting a suppressed or undeveloped apophysis.¹

2. The Entosternite of Scorpions.

The variations that affect the entosternite of Scorpions are principally correlated with the compression, antero-posterior or lateral as the case may be, of the exoskeletal metasternite. In *Palamnæus thorelli* the "body" of the entosternite consists of an irregularly transversely oblong plate. From its anterior angles rise the anterior cornua, which give off muscles to the appendages along their outer edge, and present a frayed or ragged appearance when cleared of these tissues (see fig. 20, Pl. 14). On its underside the plate dips down on each side of the nerve-cord, and passing and fusing beneath it forms a complete and rigid ventral ring through which the nerves pass backwards into the mesosoma. The lower portion of this ring gives off in front

¹ In his paper on the anatomy of *Limulus polyphemus* ('Trans. Linn. Soc.,' xxviii, 1873), Owen states (p. 469) that the entosternite of this species is furnished with a pair of "sclerous processes" which diverge from "near the fore-part of the dorsal surface," and reference is made to fig. 5 on pl. xxxviii, which is an acknowledged copy of Van der Hoeven's figure of the entosternite of *L. moluccanus*. Yet the original figures with which Owen's paper is illustrated are all, apparently, taken from examples of the American form (*L. polyphemus*). Hence it is difficult to account for his overlooking the presence of the two pairs of processes in this species. It may also be remarked in passing, though the lapsus is of no great moment, that the statement in the foot-note to p. 462 that "the species which he [Van der Hoeven] dissected was the rapier-tailed Molucca crab (*Limulus rotundicauda*, Latr.)" is an error.

a median process which divides into a pair of diverging tendinous apophyses. From the sides of the neural ring externally spring muscle-supporting processes. The muscles rising from the posterior of these processes are extended laterally and dorsally to become attached to the sides and roof of the body-cavity, forming, with associated connective tissue, a great muscular sheet or "diaphragm" which separates the cavity of the prosoma from that of the mesosoma. Inferiorly this partition is completed by muscles which run from the posterior side of the lower edge of the neural canal to the floor of the body-cavity. In the middle line above the "body" of the entosternite the connective tissue of this muscular sheet is perforated by two foramina; the inferior gives passage to the alimentary canal, the superior to the aorta.

In addition to the muscles already mentioned, three pairs of dorso-ventral muscles arise from the entosternite. Those of the posterior pair are attached to the underside of the tergite of the genital somite behind the diaphragm, and to the posterior side of the entosternite in front of it. Hence in their passage from above downwards they pass through the diaphragm. The median pair extends from the aortic foramen in front of the diaphragm to the posterior border of the upper surface of the "body" of the entosternite. Just in front of their inferior points of attachment spring those of the anterior pair, which, rising vertically, meet in the middle line above the aorta, before attaching themselves to the underside of the carapace.

As might be expected, the entosternite of this species agrees in all essentials with that of *Palamnaeus indus* (= *Buthus cyaneus*) as described and figured by Lankester (6) and Beck (1). In a general way the entosternite of all Scorpions is formed on this plan. In minor particulars, however, there is considerable structural variation. In species with the metasternite antero-posteriorly compressed, the body of the "entosternite" becomes shorter as compared with its length, as shown in the figure of that of *Iurus*

dufourei, one of the *Vejoidea*. In this species the lateral crests which arise from the anterior cornua are better developed than in *Palamnaeus*, and the homologue of the solid lateral process of the latter is less solidified and rigid. Moreover the anterior process from the neural arch appears to be undeveloped (Pl. 14, fig. 21).

In *Hadruroides charcasus*, a member of the same family as *Iurus*, but with the sternum showing a markedly greater degree of antero-posterior compression, the "body" of the entosternite is relatively much shorter than in the last-named genus, though in other respects the entosternites of the two are very similar.

The process of reduction in the size of the entosternite by longitudinal compression is carried to an extreme in the *Bothriuridae* (olim *Telegonidae*), where the sternum is reduced to a transversely linear sclerite wedged in between the genital operculum and the coxæ of the appendages of the fourth pair (= second walking leg). In *Bothriurus bonariensis* (see Pl. 14, fig. 22) the portion of the body of the entosternite which forms the roof of the neural canal is reduced to a narrow transverse bar. This modification seems to have been accompanied by the disappearance of the anterior pair of dorso-ventral muscles; those of the second pair pass up to the aortic foramen without fusing with the diaphragm. As in the genera of *Vejoidea* examined, the subneural process is apparently absent in the *Bothriuridae*. The structure of the entosternite in this family bears out the view I have elsewhere expressed that these scorpions are a specialised offshoot of the *Vejoidea*.

In the *Buthidae*, which are characterised by a triangularly compressed sternum, the entosternite shows unmistakable signs of lateral compression, the "body" being reduced to a longitudinal bar, from the posterior extremity of which, and rather between than behind those of the first pair, rise, in juxtaposition, the dorso-ventral muscles of the second pair. The lateral crests are well developed, as in the *Vejoidea*, and the subneural arch is furnished with a median process

ending in two fan-shaped apophyses similar to those of *Palamnaeus*, but stouter.

These characteristics are illustrated in the figure of the entosternite of *Centruroides margaritatus* (Pl. 14, fig. 24), which may be taken as fairly typical of the entosternite of *Buthidae* in general.

Schimkewitsch (10) gives a figure of the entosternite of *Androctonus bicolor*, which is quite unlike this plate in any member of the *Buthidae* I have examined. Presumably the form he names *A. bicolor* is the thick-tailed, dark-coloured species from *Transcaspia* which Olivier called *crassicauda*. In examples of this species the entosternite closely resembles that of *Centruroides margaritatus* (see Pl. 14, fig. 24), having the same narrow median longitudinal ridge and large lateral crests and the same narrow, nay, even narrower bar, with broad, fan-shaped apophyses running forwards from the subneural arch. Yet Schimkewitsch represents the supra-neural arch as a transversely oblong plate as wide in proportion to its length as in *Hadruroides*, furnished with lobate lateral projections, and a very broad subneural process with unexpanding apophyses. An entosternite of this description should belong to some species with a broad and short pentagonal entosternite.

Speaking of the entosternite of the scorpions, Bernard (3) says that its points of attachment "to its parent cuticle correspond with the points of origin of the entosternite of *Galeodes*,"—that is to say, to the integument immediately above the preaxial surface of the coxa of the fourth prosomatic appendage, or, as he elsewhere (4) expresses it, between the third and fourth segments. In *Palamnaeus thorelli*, the species examined by Bernard, I find that the anterior bar of the entosternite has a fibromuscular attachment to the in-projecting anterior rim of the coxa of the fourth appendage (second leg). But I could not satisfy myself that there was any union with the adjacent integument,—certainly there was none such as to justify the speaking of the integument as the "parent" of this bar

of the entosternite. Bernard also homologises the anterior bars of the scorpion's entosternite with the second pair of ventral apophyses which are affixed to the sternum opposite the base of the third prosomatic appendage (first walking leg) in the spiders. Since, however, these bars in the scorpion give attachment diagonally to great muscles which supply the second and third appendages (chela and first leg), it seems far more likely that they represent the anterior bars of the entosternite of the spiders, *Thelyphonus*, etc., which are similarly continuous with the muscles supplying these appendages (Pl. 14, fig. 20).

3. The Entosternite of the Pedipalpi (*Thelyphonidæ*, *Phrynidæ*).

In the Pedipalpi two types of entosternite are found, one characteristic of the *Urotricha* (*Thelyphonidæ*), the other of the *Amblypygi* (*Phrynidæ*). In the *Thelyphonidæ* the main portion of the plate is longitudinally oblong in shape. It is perforated mesially by two foramina, an anterior large and oval, and a posterior relatively small and circular. The two are separated by a transverse bridge; a similar bridge separates the anterior foramen from the pharyngeal notch. Near the edges of the upper surface of the right and left bars forming the external framework of the pharyngeal notch and of the foramina, rise five pairs of tendinous processes which are affixed by muscular fibres to the underside of the carapace. The first rises at the extremity of the anterior cornu, the second just in front of the anterior bridge. The latter apophysis is bifid and projects inwards, backwards, and upwards towards the central depression of the carapace. The others take a more lateral direction. The third rises close to the second and a little behind the anterior bridge; the fourth just behind the middle of the large foramen; the fifth on a level with the smaller foramen. Below the latter may be seen a bifid tendinous crest running downwards and outwards. Behind this point the entosternite

is laterally constricted, then expands into a subcircular softer plate, to the ragged edge of which are fastened many muscles passing to the pregenital somite and to the appendages of the sixth pair. Sometimes at least there is, on the upper side of this plate, a pair of short processes which serially repeat apparently the longer tendons of the anterior part of the entosternite. From the underside in front arise two pairs of processes, the first passing from the anterior extremity of the cornua to the coxæ of the chelæ, the second to the prosternum from a point on a level with the anterior bridge (Pl. 13, figs. 2, 9).

The figure and description of the entosternite of *Thelyphonus* published by Laurie (8) do not agree with this organ in the species examined by myself. The anterior three pairs of dorsal processes and the two pairs of ventral processes, as well as the lateral crest, are omitted from the figure and unmentioned in the text; and I find no process projecting from the sides of the posterior lobe such as he represents and describes. So, too, is the figure twice published by Schimkewitsch (9, 10) from a sketch by Tarnani and copied by Bernard (3) unlike, in certain particulars, the entosternites of the *Thelyphonidæ* I have dissected, although resembling them in general form and in the number of the processes. For example, the bifid process numbered *Sa.* in my drawing is represented as rising from the side of the anterior bridge slightly behind the level whence the processes numbered *2tg.* diverge; and the processes numbered *3tg.* spring further back in line with the posterior bridge, not just behind the middle of the larger foramen, as shown in my drawing. The lateral crest, too, was apparently unnoticed. Considering the uniformity in the structure of the prosoma throughout the family *Thelyphonidæ*, it seems hardly probable that these discrepancies are due to specific differences between the specimens examined. I find a practically complete uniformity of structure in the entosternite in species of the genera *Thelyphonus*, *Hypoctonus*, and *Mastigoproctus*.

The entosternite of the *Amblypygi* is very different from that of the *Urotricha*. The pharyngeal notch is semi-circular and the anterior cornua large. Each bears a pair of dorsally directed apophyses near the apex, also one on the underside, which dips down beneath the pharynx, and one above, at the base, which projects upwards and inwards. The body of the plate itself is wide, narrowed posteriorly, and solid, i. e. without foramina. Near its lateral border on each side arise four apophyses which extend upwards and outwards to be inserted by means of muscular bundles to the under surface of the carapace. The first is very slender; the second and third are approximated at the base; the fourth is the stoutest. These four spring from a common ridge beneath which the edge of the entosternite runs out externally into a short angular crest to contribute support to the great appendicular muscles (Pl. 13, fig. 3).

It would be unfair to criticise the figure of the entosternite of "*Phrynus*" given by Bernard (3), because "the preparation was accidentally destroyed before the drawing was completed." Four pairs of dorsal apophyses are represented, but I cannot satisfactorily homologise them with the six pairs shown in the figure here published (Pl. 13, fig. 3). It is stated, moreover (op. cit., p. 20), that this plate has "only one attachment to the ventral surface, and that is to the intersegmental membrane between the second and third pairs of limbs corresponding with the first pair of apodemes forming the entosternite in *Mygale*." It is true that there is only one pair of ventral processes, and that they represent the similarly situated processes in *Mygale*. They are not attached, however, in the position Bernard states, but to the coxa of the second appendage, the point of their insertion appearing as a horny subcircular patch on the soft membrane below the mouth, when these appendages are pulled apart and examined from the front.

4. The Entosternite in the *Araneæ*.

In typical members of the *Araneæ* the entosternite closely

resembles that of the *Amblypygi* in general form and in many structural details. In *Epebopus murinus* and other mygalomorphous spiders of the family Aviculariidae it is a longitudinally oval imperforated plate, with large anterior cornua bounding the pharyngeal notch. The upper side is furnished with four pairs of dorsally directed tendinous processes, arising, as in *Phrynus*, from a common ridge. Below this the edge of the plate runs out externally into angular processes which afford attachment to some muscles of the legs. From the underside four pairs of processes pass downwards to meet the sternum; the first pair arising from the anterior cornua and running to the bases of the appendages of the second pair, immediately behind the prosternum; the second, third, and fourth radiating from a common median excrescence, behind the pharyngeal notch, and reaching the sternum opposite the third, fourth, and fifth appendages. Remnants of a similar apophysis corresponding to the sixth appendage, but failing to reach the sternum, are traceable near the posterior end of the entosternite. The chief difference between this entosternite and that of the *Amblypygi* lies in the presence of the ventral apophyses corresponding to the four posterior pairs of prosomatic appendages.

The figure of the entosternite of a *Mygale* given by Bernard (3, figs. 3 and 5), and taken from a specimen in the College of Surgeons' Museum, is diagrammatic. It is to be noticed, however, that the ventral processes of the first pair are correctly represented as fused in the middle line. The entosternites of the species examined by Lankester (5, 6)¹ and Wasmann (11) agree closely with that of *Epebopus murinus*.

In the great majority of the Mygalomorphæ the entosternite is in the main like that of *Epebopus*, retaining the four dorsal and the four ventral apophyses, the points of attachment of the latter being visible on the external

¹ In the figure published in the second of the two works enumerated above the dorsal side is by an oversight represented as the ventral, and vice versâ.

surface of the sternum as the so-called sigilla. In many genera there is a tendency for the posterior pair to increase in size and shift their point of insertion from a submarginal to a subcentral position. This is particularly noticeable in the so-called "trap-door" spiders, where the muscles and appendages of the prosoma are specialised for fossorial work.

In a few genera, e. g. *Atypus*, *Eriodon*, and *Actinopus*, all the four pairs of ventral apophyses have moved from the margin of the sternum towards its centre, the convergence reaching an extreme in *Actinopus*, where their points of attachment meet in the middle line, forming the well-known rosette or star-shaped sternal impression characteristic of this genus. The union of these four apophyses on each side with one another and with their fellows of the opposite side results in the formation of a solid plate beneath the nerve mass, which is thus enclosed, as it were, in a basket, the lateral nerves to the limbs passing out through the spaces between the upright portion of the apophyses. From the middle of the anterior border of this ventral plate a short median process runs forward, forming the median unpaired lobe of the rosette-like impression on the outer side of the sternum.

In *Atypus* the four apophyses retain their primitive distinctness, and are arranged on the underside of the entosternite in the form of a circle, following the curvature of the pharyngeal notch. A fibrous strand runs forward from the anterior apophysis to the prosternum.

A reduction in the number of ventral apophyses takes place in the typical genera of the Otenizinae, e. g. *Pachylomerus*, *Stasimopus*, and of the Idiopinae, e. g. *Acanthodon* and *Heligmomerus*. In *Pachylomerus* the first and fourth apophyses persist, the second and third disappear. *Stasimopus* resembles *Pachylomerus* in this particular, but differs in that the apophyses of the anterior pair fuse across the middle line to form a complete collar round the nerve mass. In *Acanthodon* the first apophysis is retained as in all the Mygalomorphae, and the second

and third also as slender pillars with a marginal attachment to the sternum, but the fourth pair has vanished. In some of the genera of this group, e. g. *Stasimopus* and *Pachylomerus*, an additional apophysis is found on the dorsal side arising from the crest just behind the second apophysis from the anterior end, and directed inwards. Indications of a similar tendon are also observable behind the next succeeding apophysis, and in *Acanthodon* similar supplementary tendons are observable behind the posterior two pairs of apophyses.

In an immature specimen of *Liphistius* I find the four normal dorsal apophyses of exceptional thickness, and representatives of the two supernumerary apophyses that occur in *Stasimopus*, well developed. The entosternite in this specimen, however, perhaps on account of its immaturity, has no ventral apophyses extending to the sternum, although the muscular scars are visible at the sides of this plate. This absence of ventral apophyses is full of interest, on account of its repetition in the *Arachnomorphæ*, with which *Liphistius* has other features in common (Pl. 13, fig. 7).

On the structure of the entosternite in the *Arachnomorphous* spiders (olim *Dipneumones*) my observations have not been far extended. A few examples of genera belonging to widely separated families have been examined, however, without the discovery of any very marked differences in the structure of this plate. In all there are four pairs of dorsal apophyses corresponding exactly to those of the *Mygalomorphæ* and *Liphistius*, and in all, except *Filistata*, an additional pair arising, one on each side, between the normal second and third pairs, and directed obliquely inwards and backwards. This represents, no doubt, the muscle, sometimes with a tendinous base, which arises in the same position in some of the *Mygalomorphæ*, e. g. *Pachylomerus*. In many strong-legged species, such as *Lycosa*, *Ctenus*, and *Kresus*, it is noticeable that the dorsal tendons are broad and divided distally into two branches. The extension of this split to the root of the tendon would give rise in each instance to two

complete and distinct apophyses. This cleavage appears to have taken place, probably once, possibly twice, in the case of the entosternite of *Phrynus*.

In none of the *Arachnomorphæ* have I found ventral apophyses extending to the sternum, such as are found in all the *Mygalomorphæ*. The underside of the entosternite of *Lycosa ingens*, however, is furnished in its anterior half with a high median crest, from which five short and slender tendons arise on each side. These tendons appear to be homologous with the five inferior tendons seen in *Ephebopus* (fig. 13) and other genera of *Aviculariidae*. In the latter, however, only three pairs spring from a common centre, the first lying far forward; the fifth, often obsolete, far backwards.

The diagrammatic transverse section of the entosternite, with its associated muscles, of the *Aranææ*, figured by Schimkewitsch (10), shows on each side two dorso-ventral muscles, a lateral muscle, and two that pass to the legs, an external or elevator of the trochanter (second segment), and an internal (the depressor of the coxa), which passes ventrally to an entapophysis between the sternum and the base of the leg,—all rising from distinct apophyses. The last-named muscle is, I believe, the ventral portion of the tergo-sternal muscle, and the pair of dorsal muscles on each side represent the tergal portion cleft to the base.

5. The "So-called" Entosternite of the *Solifugæ*.

The "so-called" entosternite of the *Solifugæ*, both in its structure and attachment, is quite unlike the true entosternite of other Arachnids. It consists of a pair of stout, rigid chitinous pillars united, with or without articulation, to the narrow prosternal plate, wedged in between the coxæ of the third pair of appendages. From the prosternum it extends transversely along the narrow strip of integument joining the juxtaposed coxæ of the third and fourth pairs of appendages. Internally the two pillars, running obliquely or subvertically

backwards from their base, converge and meet, without actual fusion, in the middle line, expanding beneath the alimentary canal to form a somewhat saddle-shaped enlargement for muscular attachment. This structure is supported beneath by a pair of slender chitinous rods which rise, one on each side, from a point on the ventral integument of the fourth somite close to the inner extremity of the tracheal stigma. In front of this entosternite there is a pair of fibrous nodules, each of which forms a centre for the attachment of five tendons, one passing backwards to be attached to a forwardly directed process from the expanded portion of the entosternite, a second passing vertically downwards towards the point of attachment of the entosternite, a third passing forwards, a fourth obliquely downwards and outwards, and the fifth downwards and inwards to the base of the rostrum. Bernard (3 and 4) says the entosternite of *Galeodes* "rises as a pair of infoldings of the cuticle between the third and fourth segments," and his drawings represent the two pillars as attached some distance from the middle line to the external portion of the membrane between the coxæ of third and fourth appendages, no connection with the prosternal plate being shown or mentioned. The only addition to be made to his description relates to the attachment of the entosternite to the prosternal plate as mentioned above.

This attachment is of two kinds. In the case of a specimen of *Solpuga sagittaria* the continuity of the entosternite with the prosternum and the intercoxal integument is plainly indicated after clarification in caustic potash and immersion in glycerine. I can find neither articulation nor sutural line, to attest its obliteration, between the two. On the contrary, the strengthening strands of thick chitin which traverse the entosternite pass without interruption into those of the prosternum, the two forming a rigid and continuous whole. A similar state of things is shown in the figure of the skeletal elements of the prosoma of *Galeodes* given by Schimkewitsch (10).

The treatment mentioned above applied to the entosternite

of *Galeodes arabs* revealed, however, a joint between the posterior extremities of the bars of the V-shaped prosternum and the diverging pillars of the entosternite.

The question as to which of these two arrangements is the more primitive must remain unanswered until the origin of the Galeodean entosternite is settled. If, as Bernard maintains, it is nothing but an exoskeletal entapophysis, the condition of unbroken continuity with the exoskeleton, as manifested in *Solpuga*, must be regarded as the original, and the jointed condition seen in *Galeodes* the derivative. If, on the other hand, the entosternite in this order proves to be an entoskeletal element like that of other Arachnids, its fusion with the exoskeleton must be a secondarily acquired characteristic, and its separation therefrom a primitive feature (Pl. 14, figs. 26, 27).

II. THE COMPARATIVE MORPHOLOGY OF THE ENTOSTERNITE.

The evidence favouring the hypothesis that the prosoma of the primitive Arachnid was furnished with a broad segmented sternal area separating the post-oral appendages of the right side from those of the left needs no recapitulation. It may be claimed that the possession of a wide sternal area by the Amblypygous Pedipalpi and all the typical Araneæ is an archaic feature; and that, in this particular at least, these orders are less specialised than the Scorpiones, Solifugæ, Pseudo-scorpiones, and Opiliones, where the encroachment of the coxæ of the appendages, aided in the case of the first and last-named orders by antero-posterior compression accompanying the forward movement of the generative aperture, has more or less obliterated the sternal sclerites in the middle line. Although modified to a very appreciable extent in the direction of sternal suppression, the prosoma of the Uropygous Pedipalpi is more primitive than that of the four orders last named, more primitive even than that of the Amblypygi and Araneæ in the lesser constriction of its posterior somite to share in the formation of the waist.

What is true of the Pedipalpi and Araneæ is also true of the Xiphosuræ. In *Limulus* there is a relatively wide sternal area extending from the mouth to the posterior extremity of the prosoma, and strengthened by a pair of strong metasternal sclerites behind and a weakly chitinised promesosternal plate in front.

Correlated with this primitive development of the sternal area we should expect to find entosternites of a more archaic type in the Pedipalpi, Araneæ, and Xiphosuræ than in the other orders of Arachnids. This expectation is justified by the unmistakably metameric arrangement of the constituent elements of the entosternite exhibited in these three orders. On almost any theory of the origin of this plate, segmental repetition of its parts must be postulated as a primitive feature. It is obvious that this characteristic is manifested in a far greater degree in the entosternite of the three orders named than in that of the Scorpiones, Pseudo-scorpiones, or Opiliones.

A satisfactory settlement of the homologies of the several parts of the various types of entosternites already described is a matter of no little difficulty on account of the variation in number of the apophyses that arise from them. In the Araneæ the dorsal apophyses range in number from four to six. In the Phrynidæ there are six; in the Thelyphonidæ five, with indications of an additional pair on the posterior lobe of the entosternite.

In the Araneæ the apophyses in question are referable to two categories, those that are directed obliquely inwards towards the central apodeme of the carapace, and those that arise subvertically to be inserted serially along the area between its middle line and lateral border. The latter are invariably present, and invariably four in number on each side; the former are either absent or represented by one or two pairs. In the two types of entosternite presented by the Pedipalpi there is a single pair of apophyses directed inwards and backwards, arising in each case close to the base of the anterior cornua.

As explained below (p. 249), there are good reasons for supposing that the four apophyses of constant occurrence in the Araneæ represent the tergal elements of the tergo-sternal muscles of the second, third, fourth, and fifth somites of the prosoma, those of the sixth somite being undeveloped as an accompaniment of the compression this somite has suffered.

Seeing how nearly related in many particulars the Araneæ are to the Pedipalpi, it can hardly be doubted that the four apophyses in question in the Araneæ are homologous to the four that project laterally in the Thelyphonidæ, the fifth pair which is suppressed in the Spiders being retained in a rudimentary state by the Pedipalpi. In the case of Phrynus the question is complicated by the presence of an additional apophysis on each side, making a total of five. The posterior of these might be held to represent the apophysis which is missing in the Araneæ and rudimentary in Thelyphonus; but since the last prosomatic somite in Phrynus is compressed in its sternal portion almost to the same extent as in the Araneæ, and since even in Thelyphonus, where this somite retains its more primitive condition, the apophysis is scarcely developed, it seems more probable that this apophysis is also undeveloped in Phrynus, and that the fifth apophysis in this genus actually corresponds to the fourth in the Spiders and Thelyphonus. The likelihood of the truth of this view is enhanced by the basal juxtaposition of the third and fourth apophyses in Phrynus, which forcibly suggests their common origin from a tendon representing the third apophysis of the Spiders and Thelyphonus secondarily subdivided into two. The possibility of the subdivision of these apophyses is clearly shown in the case of many Araneæ, such as Ctenus and Lycosa where they are deeply cleft, in Acanthodon where those of the third and fourth pairs are double down to the root, and in Thelyphonus where the apophysis rising from the upper side of the anterior cornu gives off a secondary branch towards the middle line.

Cleavage of primary single tendons may account for the presence of the one or two pairs of supplementary tendons

which run obliquely inwards and backwards towards the centre of the carapace. The constancy in position of these tendons in the Spiders suggests their homology throughout the order, and their origin from the second or the second and third of the larger normal apophyses. Only one such apophysis is developed in *Thelyphonus*, and this arises a little in front of the second marginal tendon, not behind it as in the Spiders. Interesting, therefore, it is to observe that in *Phrynus*—a genus in many respects intermediate between *Thelyphonus* and the Spiders—the apparent homologue of this tendon lies a little farther back than in *Thelyphonus*, a little farther forwards than in the spiders. It is also noticeable as a peculiarity in *Phrynus* that in the third tendon, which, for reasons already given, may be regarded as reduplicated, the extra branch takes the same direction as its twin. One other small structural feature bears out the homologies here suggested. This is the presence in many spiders of a transverse thickening of the entosternite just in front of the fourth marginal apophysis. The posterior bridge of the entosternite in *Thelyphonus* has exactly the same relations. Similarly the posterior margin of the pharyngeal notch in the Spiders is generally thickened, so as to suggest its correspondence with the anterior bridge in *Thelyphonus*. As for the ventral apophyses, there cannot be much doubt that the pair passing from the anterior cornua to the basal segments of the second appendages in *Phrynus* and *Thelyphonus* are the homologues of each other and of the anterior pair, which have the same origin and are affixed to the sternum close to the base of these appendages in the *Mygalomorphæ*. So, too, must the second apophysis attached to the sides of the prosternum in *Thelyphonus* represent the second apophysis attached to the sternum opposite the base of the third appendages (first leg) in the *Mygalomorphæ*. Thus it is possible to bring into complete accord the apophyses developed on the dorsal and ventral sides of the entosternites of the three orders here considered.

Scarcity of material for comparison seems to have prevented

Bernard's recognition of the homologies existing between the parts of this plate in different orders, homologies which are, at all events, fairly obvious in the case of the Pedipalpi and Araneæ. To quote his own words (4), "In the Spiders . . . the entosternite consists of four pairs of apodemes which meet in the centre, the second pair of which correspond with the entosternite of Galeodes and Scorpio, whilst the first pair is perhaps represented in Galeodes by the fibrous plate above described. In Phrynus the entosternite is difficult to unravel; it may perhaps represent only the first pair of apodemes of the spiders with secondary attachment of dorso-ventral muscles. In Thelyphonus we have a long fenestrated entosternite which may correspond with that of the Spiders; the component apodemes not, however, meeting in a point."

I venture to think that the new facts and theories concerning the entosternite of the Pedipalpi and Araneæ put forward in this essay will show that the homologies are by no means so vague and difficult to unravel as the passage quoted would lead one to suppose.

Schimkewitsch (10) terms the apophysis that rises from the upper side of the anterior bar in Thelyphonus a dorso-ventral outgrowth, and those numbered 1tg., 2tg., 3tg., and 4tg. in fig. 2, Pl. 13, as lateral outgrowths, homologising the latter apparently with the lateral crest, and the former with one of the dorsal apophyses in the Araneæ. I cannot think this interpretation correct in view of what obtains in the entosternite of Phrynus. The Pedipalpi and the Araneæ are so very closely related that the conclusion as to the homology between the apophyses of the entosternite appears to me inescapable.

The entosternite of Limulus forcibly recalls that of Thelyphonus. The anterior bars correspond in the two. Following these in L. polyphemus come the two long, slender apophyses running out towards the bases of the third and fourth appendages, and representing, I believe, the dorsal portions of the tergo-sternal muscles of the corresponding somites. A comparison between these and the second and

third pairs of lateral processes seen in *Thelyphonus* and Spiders is obvious, and is fortified by the evidence favouring the view that in the spiders at least these processes correspond with the second and third pairs of post-oral appendages. It is only necessary to homologise the muscle-bearing stump in *Limulus* with the fourth lateral process in *Thelyphonus* and the Spiders, and the strong postero-lateral apophyses in the entosternite of *Limulus* with the vestigial processes on the posterior lobe of the entosternite in *Thelyphonus* to complete the parallel. On the underside similarity between the entosternite of *Limulus* and the Arachnomorphous Spiders is to be found in the absence of ventral apophyses, with the exception that in *L. polyphemus* a single abbreviated pair is present at the posterior end of the plate exactly as in some of the Mygalomorphous Spiders, e.g. *Ephobopus*.

The entosternite of Scorpions has been so affected by the compression of the prosoma that it is not easy to bring it into exact line with those of the orders hitherto considered where a more primitive condition persists. That the anterior bars framing the pharyngeal notch are comparable to those of *Limulus*, the Pedipalpi, and Araneæ hardly admits of a doubt (Lankester, 5, 6, and 7). Similarly the lateral tendinous crest supporting the leg-muscles, and so well developed in the Buthidæ and Vejovidæ, forcibly recalls that of the Araneæ. But the dorsal apophyses which form so conspicuous a feature in the entosternite of the Pedipalpi and the Araneæ remain undeveloped. They are represented by the two pairs of dorso-ventral muscles which lie in front of the diaphragm, those of the third pair which perforate this partition being usually regarded as the tergo-sternal muscles of the genital or first somite of the mesosoma.

To which three of the four or five pairs of dorsal apophyses present in the Araneæ and Pedipalpi do these two pairs in the scorpion correspond? Probably, I think, to the fourth and fifth pairs,—that is to say, to those that belong to the fifth and sixth segments of the prosoma. This homology is suggested by their position at the posterior extremity of the prosoma,

and by the fact that the somites in question have retained their sternal elements, and are therefore, in that particular at least, less modified than the somites in front, in which the sterna have disappeared. If, then, we suppose that the pharyngeal notch in the scorpions has been prolonged backwards almost as far as the position of the posterior transverse bridge in *Thelyphonus*, or as the corresponding thickened ridge in the *Araneæ*; that the anterior three pairs of apophyses have been suppressed upon the two cornua; that the lateral tendinous crests represent the similar crests in the *Araneæ* and *Pedipalpi*, those of *Palamnæus* in particular recalling those of *Thelyphonus*,—it is evident that the entosternites in the orders now under discussion are more alike in reality than appears at first sight on the surface. Furthermore, if we suppose that representatives of the muscles which radiate from the margin of the posterior lobe of the entosternite in *Thelyphonus* extended dorsally and laterally to meet the walls and roof of the prosomatic space, leaving a channel in the middle line for the transmission of the aorta and the alimentary canal, it is possible to bring even the diaphragm into harmony with parts already existing in the *Thelyphonus*. In short, strip away the apophyses lettered *1tg.*, *2tg.*, *3tg.*, and *sa.* in the figure of the entosternite of *Thelyphonus*, remove the anterior bridge (*a. b.*), and fill up the posterior foramen, and the homology of the remainder of the plate with the supra-neural portion of the entosternite in the Scorpions becomes obvious. The annexation by the entosternite of the tergo-sternal muscles of the genital somite probably took place before the upgrowth of the posterior flap shut off the cavity of the prosoma from that of the mesosoma; and this consideration points to the formation of the diaphragm after the suppression of the pregenital somite and after the forward movement of the ventral area of the genital somite, which brought its tergo-sternal muscle into contact with the entosternite.

The origin of the dorsal portion of the diaphragm in this way from a pair of upgrowing muscular flaps embracing the

alimentary canal and aorta is attested by the persistence of the divisional line between its right and left halves. They are merely united by a strip of connective tissue, perforated above and below by the aortic and alimentary foramina, which must be regarded as the sole remnants of the open space which originally separated the right and left portions of the flap from one another.

The neural ring in the Scorpions has its counterpart in *Actinopus*, even to the development of an anterior median process. It may have arisen in the same way by the fibrous solidification and subsequent subneural fusion of the ventral moiety of a pair of tergo-sternal muscles. If so, the view that only one such pair of muscles is involved in its construction, and that that pair belonged to the sixth somite of the prosoma, is suggested by the absence of lateral perforations in the sides of the neural arch for the exit of nerves to the appendages, and by the situation of the ring behind the point whence the nerves to the appendages of the sixth pair diverge. Equally well, however, may the sides of the canal have arisen from the downward growth of the lateral portion of the underside of the posterior portion of the entosternite.

Lankester (6) suggests that the lateral process marked *cr.* in the figure of the entosternite of *Palamnæus* (Pl. 14, fig. 20) corresponds to the antero-lateral processes of the entosternite in *Limulus*. More likely, I think, is it that this process is the thickened and solidified representative of the posterior part of the crest developed (Pl. 14, figs. 21, 24) in *Centruroides* and *Iurus*, and finds its homologue in the similar crests in *Thelyphonus*, and not in the dorsal apophyses, to which I believe the two processes in *Limulus* are comparable. Nor can I agree with the opinion of Schimkewitsch (10), that the lateral processes he finds on the entosternite of the Scorpion named *Androctonus bicolor* (see p. 231) are the homologues of the processes I have numbered *1tg.*, *2tg.*, and *3tg.* in *Thelyphonus*.

III. THEORIES OF THE ORIGIN OF THE ENTOSTERNITE.

As long ago as 1881 Lankester (5), when describing the entosternite of *Limulus*, said it may be regarded as an enlargement and interlacing of the respective tendons of the muscles which are attached to it. By implication a similar origin was predicated of the entosternites of Scorpions and Spiders. This opinion was accepted by Schimkewitsch (9, 10) in the case of the entosternite of Scorpions, Spiders, and other air-breathing Arachnids, and for that of *Limulus* by Bernard (3), who, however, regarded it solely as a derivative of the ventral longitudinal muscle-bands. Bernard's views as to the origin of the entosternite in the terrestrial Arachnids, which he considers to be in no way related to *Limulus* and its extinct allies, will be referred to later on.

It appears to me that the evidence in favour of Lankester's view of the mode of production of this plate in both groups of Arachnids is overwhelming; a comparative study of the entosternites in this class precludes, to my mind, any other hypothesis as to their source.

What muscles, then, have taken the largest share in their formation?

There is reason to believe that the prosoma was originally supplied with five pairs of tergo-sternal (dorso-ventral) muscles serially repeating those of the opisthosoma, and passing vertically from the under surface of the carapace to be inserted ventrally on the sternum close to the points of articulation of the post-oral appendages. There were also a dorsal and a ventral pair of longitudinal muscles traversing the prosoma from end to end (see Lankester, 7).¹ With the

¹ "The simple musculature in the ancestor consisted of—(1) a pair of dorsal longitudinal muscles passing from tergite to tergite of each successive segment; (2) a similar series of paired longitudinal ventral muscles; (3) a pair of dorso-ventral muscles passing from tergite to sternite in each segment; (4) a set of muscles moving the coxa of each limb in its socket. The confluence of the prostomium and the six anterior tergites to form a prosomatic carapace, as well as the specialisation of the six pairs of appendages of the prosoma, was common to the ancestors of both *Limulus* and *Scorpio*. This modi-

welding together of the external skeletal elements to form a compact inexpandible whole, the function of these muscles as dilators and contractors of the prosoma would cease, leaving them available for other purposes if required.

The fusion of tergites to form a carapace, accompanied no doubt by the partial or complete cessation of function of the longitudinal and vertical muscles, took place, as may be seen, in the Trilobites, before the five pairs of post-oral appendages of the prosoma were set apart as the exclusive organs of locomotion and prehension. This specialisation, demanding an increase in the size and strength of the limbs in question, would be advantageously accompanied by an increase in the area for the attachment of the enlarged and subdivided muscles that control them. This area might be supplied, in the first instance, by the fibrous solidification of the central portion of the tergo-sternal muscles, aided perhaps by that of the adjacent portion of a longitudinal muscle on each side passing from the anterior to the posterior extremity of the prosoma above the nerves radiating to the appendages.

Does the structure of the most primitive types of entosternite known to us furnish justification for the opinion that they have originated in the manner here suggested? A good deal may be said, I think, in favour of an affirmative answer to this question.

The points of attachment of the tergo-sternal muscles of the opisthosoma are generally apparent enough externally, both on its dorsal and ventral walls. On the prosoma they are usually much less apparent. In the Mygalomorphous spiders, however, the sternum is typically marked with four pairs of "scars," the so-called "sigilla," one on each side close to the proximal end of the coxa of the second, third, fourth, and fifth appendages (i. e. the palpi and first three pairs of legs). The position and nature of these scars at

fication of form and specialisation of body regions entailed a corresponding modification of the muscular system. The dorsal and ventral longitudinal muscles of the prosoma were suppressed. The muscles of the prosomatic limbs acquired large size and became subdivided."

once suggest their correspondence with the similarly placed scars upon the sterna of the opisthosoma in, e. g., *Phrynus*, which admittedly indicate the ventral attachments of the tergo-sternal muscles. Dissection, however, shows that the scars on the sternum are the points of insertion, not of muscles, but of the tendinous processes which project downwards from the lower surface of the entosternite. These tendinous processes are, I believe, the solidified ventral moieties of the primitive prosomatic tergo-sternal muscles. Apart from other considerations, their muscular origin is attested by their representation in the *Arachnomorphæ* (e. g. *Ctenus*) by muscles passing from the lower surface of the entosternite and affixed by a fibrous strand to corresponding points on the sternum.

The dorsal moieties of these same muscles are represented, I think, by the paired tendinous apophyses springing from the upper side of the entosternite and passing into muscular fibres which fasten them to the lower side of the carapace. There is never a sternal scar near the base of the sixth appendage in the *Mygalomorphæ*, and no apophyses, either dorsal or ventral, of any appreciable length, corresponding to this limb, on the entosternite. Short chitinous ridges are observable, however, on this plate in the appropriate positions. These considerations suggest the suppression of the fifth pair of apophyses as a concomitant, no doubt, of the constriction as the last somite of the prosoma.

The acceptance of this view of the nature of the dorsal and ventral processes of the entosternite carries the conclusion that a large part of this plate in the *Araneæ* results from the tendinous solidification of five pairs of tergo-sternal muscles.

Evidence that a share in the formation of the plate has been contributed by at least one pair of longitudinal muscles is supplied by the following facts. Apart from the appendicular muscles, which originally took a transverse direction, both the anterior and posterior extremities of the entosternite afford support to muscles; those from the anterior bars passing forwards to the front wall of the prosoma,

those from the posterior extremity running backwards into the opisthosoma in continuity with the longitudinal muscles of this region. This is well shown in *Limulus* (Benham, 2); in *Epeira* by Schimkewitsch, and in the specimen of *Atypus* figured in Pl. 14, fig. 17. Again, in the Thelyphonidæ, the anterior two thirds of the entosternite, apart from the dorso-lateral apophyses, consist of a pair of stout parallel longitudinal bars, united by an anterior and a posterior transverse bridge, the posterior lobe alone consisting of an undivided subcircular plate.

The solidification of these muscles was no doubt brought about to afford a firm support for the muscles of the prosomatic appendages. To resist the action of these muscles, which would tend to draw the bars asunder, tendinous bridges were developed across the middle line serving to hold the entire structure in place. As a later development in the Araneæ and the Amphygyi, the intervals between the bridges were filled in and the projecting marginal angular crests so characteristic of the entosternite of the Araneæ were formed to increase the attachment-area of the leg-muscles. Latent potentiality for transverse fusion between the originally separated right and left halves of the entosternite may be inferred from the fusion of this nature that has actually taken place between the ventral apophyses in *Actinopus*.

It is possible that the longitudinal muscles have not played so important a part in the formation of the entosternite as here suggested. The entosternite may be almost equally well derived on theoretical grounds from dorso-ventral and crural muscles alone, the anterior bars forming the pharyngeal notch resulting from the fusion of the tendons of the dorso-ventral muscle of the second somite with those of the appendages of the second and third somites, and the longitudinal direction of the bars being assignable to the forward movement of the second appendage pulling the tendons in a semicircle round the pharynx and brain, the notch thus formed becoming deeper and deeper to accommodate itself to the concomitant backward movement of these organs.

The opinion put forward by Lankester in 1881 (5) that the entosternite was formed by the enlargement and interlacing of muscular tendons was modified in 1885 (7) by the further suggestion that the prosomatic and smaller posterior (mesosomatic) entosternites are merely the original subepidermic connective tissue of the sternal surface of the segments in which they occur, which has become thickened and cartilaginous, and, in the case of the prosoma, has been at the same time floated off, as it were, from the sternal surface, taking up a position deeper, that is to say nearer the axis of the animal than that which it originally occupied. "And, again, in both *Limulus* and *Scorpio* the prosomatic entosternite or plastron represents the mid-sternal area of several segments fused, probably in both cases of all the prosomatic somites, though possibly in *Scorpio* the first segment is not included." The position of the entosternite above the nerve-cord is explained on the hypothesis that the detachment of the mass of connective tissue from the sternal surface occurred at a period when the nerve-cords were still quite lateral in position, their union taking place after the flotation.

This hypothesis assigns an immense antiquity to the entosternite, an antiquity dating back probably to the Trilobitic stage of Arachnid phylogeny, possibly earlier still. But supposing that the entosternite owes its origin to the detachment of subneural fibrous thickenings of connective tissue, a later phylogenetic stage can be ascribed to it by assuming its derivation from paired thickenings which floated off on each side of the united nerve-cords, and subsequently fused with one another both transversely and longitudinally to form a gate-like framework beneath the digestive tract. May be the fenestration of the entosternite of *Thelyphonus* is a survival of this early stage. But whether the entosternite had its origin in subneural thickenings of this nature, and, if so, the manner and purpose of their assumption of their present position, or whether it was derived from the fibrous solidification of muscular and connective tissue in the

mid-region of the prosoma, as I am inclined to believe, are questions which must for the present be left unsettled. The evidence we possess that at least the dorsal and ventral processes of the entosternite in the spiders are modified muscular tendons seems to make it unnecessary to look elsewhere for the source of the formation of the entire plate.

This conception of the origin of the entosternite from muscular and connective tissue differs entirely from that held by Bernard (3, 4), who would derive this plate in all terrestrial Arachnids from integumental apodemes and segmental constrictions. It may be inferred from what he says about the entosternite of *Mygale* that he regards its four pairs of dorsal and ventral processes as the remains of integumental infoldings marking the line of union of the originally separated tergal and sternal elements of the prosoma.¹ He adds, "The shape of the whole fused mass has been, no doubt, much altered by the action of muscles, but its essential nature as a fusion of metamerically recurrent apodemes cannot be mistaken" (3, p. 20). In his paper on the morphology of the *Galeodidæ* (4, p. 327) he says that in the *Pedipalpi* and *Araneæ* "four pairs of dorso-ventral muscles have been retained, more or less modified, as the dorsal attachments of the entosternite, and are now largely fibrous; they suspend the entosternite and separate the alimentary diverticula in the typical manner." Hence may be inferred the admission that part at all events of the dorsal processes of the entosternite have been derived from dorso-ventral muscles. If part, why not the whole of the dorsal process? And if the dorsal process represents the part of the muscle above the entosternite, what reason is there for refusing to

¹ Bernard states that the original distinctness of the terga of the carapace in the *Araneæ* is shown by the furrows on its dorsal surface. These furrows are in reality the external indications of the radial arrangement of the great dorsal appendicular muscles, and mark the lines of attachment of the muscles rising dorsally between them from the entosternite. If the grooves indicated the union of tergal plates, such plates should be more clearly defined in the embryo, but so far as I am aware the carapace of spiders at no period of its development shows division into separated tergal plates.

regard the ventral processes as the representatives of the ventral moieties of these same muscles?

Bernard's hypothesis involves the assumption of a degree of dislocation and rearrangement of muscles and integumental apophyses for which it is difficult to find justification. I can discover no evidence that the sternal scars of the *Mygalomorphæ* and the ventral processes of the entosternite which rise from them are the remains of integumental dissepiments. If this were the case we should expect to find an intimate unseverable union between the sternum and the processes in question. No such union exists. The expanded extremities of the processes may be readily detached, the extent of their union with the sternum being quite compatible with the theory of their muscular origin, but hardly reconcilable with that of their derivation from ectodermic ingrowths.

The basis of Bernard's hypothesis is to be found, firstly, in the structure and relations of the so-called entosternite of the *Solifugæ*, which is shown by its histology and union with the integument to be an ectodermic entapophysis; secondly, in the assumption that this skeletal piece is the morphological equivalent of the entosternites of other *Arachnids*; thirdly, in the conception that the *Solifugæ* retain a more archaic type of prosoma than that of the other orders of this class.

Assuming the truth of the propositions here stated or implied, the conclusion Bernard draws as to the ectodermic origin of the entosternite in the *Spiders*, etc., necessarily follows. But from what is known of the structure and development of the entosternite in the two orders there is little doubt that the first proposition is true, and that the second is untrue. As for the third, it is a matter of opinion depending upon the standpoint from which the morphology of the *Arachnida* is regarded.

The available evidence is, in my opinion, decidedly in favour of the view that the "entosternite" of the *Solifugæ* must be regarded as a post-oral entosclerite comparable to the crescentic pre-oral entosclerite of the *Scorpions*. But

if there are any who see in it the homologue of the Scorpion's entosternite, they will remember that chitin has been shown (Lankester, p. 6) to be present in the entosternites of Scorpio, Mygale, and Limulus, and will realise the possibility of the formation of the rigid and horny Galeodean entosternite by increased development of its chitin, followed or accompanied by fusion with the exoskeleton of the second post-oral somite.

Briefly, then, of the three suppositions that may be entertained with regard to the "entosternite" of the Solifugæ, each points to its being a specialised, not a primitive structure. (1) If it is an entosclerite, as Bernard and Schimkewitsch maintain, it is not the homologue of the entosternite of other Arachnids, which is shown by its morphology and development to be an entochondrite, produced by the condensation of connective tissue and the fusion of muscular fibres and tendons. In this case it has functionally replaced the true entosternite, and is a recent specialisation, not a primitive structure. (2) If it is an endochondrite and the homologue of the entosternite of other Arachnids, its structural similarity to, and fusion with, the exoskeleton also attest high specialisation. (3) If it has resulted from the union of the true entosternite with a pair of exoskeletal ingrowths—if, say, the expanded portion supporting the alimentary canal corresponds with the true entosternite, and the pillars diverging therefrom to the exoskeletal elements,—the absence of all trace of union between the two, the complete continuity of their tissues, again indicate great specialisation.

The evidence in favour of the truth of the first supposition is almost strong enough to enforce its unquestioned acceptance. But whichever of the three prove consonant with fact and be ultimately adopted, the Solifugæ must be regarded as the most specialised type of Arachnid known, so far as the organ under discussion is concerned—a conclusion which is perfectly in accord with many, nay most, of the structural features of this order.

The one hypothesis of all others which, in my opinion, has least in its favour is that in the Solifugæ we find a primitive type of prosoma and a primitive type of entosternite clearly attesting the exoskeletal origin of this plate in all orders of Arachnida; and the conclusions deduced from these disputable premisses that the true entosternites have been derived from chitinous integumental apodemes is contradicted by their structure in the adult and their mesoblastic origin in the embryo.

Lastly, according to Bernard (3), the "diaphragm" of Scorpio, "like that of Galeodes, is the homologue of the great constriction between the sixth and seventh segments forming the 'waist' of other Arachnids, . . . a diaphragm or waist being typical of Arachnids." It is not at all clear how a partition like the Scorpion's diaphragm, composed of muscular and connective tissue and without exoskeletal elements, can be the homologue of an exoskeletal constriction. Analogous structures, structures with the same physiological significance, they no doubt are; but homologous, surely not.

According to Bernard's theory of waist formation, I presume the condition initiated in Thelyphonus and culminating in the Spiders, a condition which results from the constriction and reduction of the pregenital somite, preceded the condition now met with in the Scorpions and Solifugæ. In that case the "diaphragm" in these two orders must represent the pregenital somite, insunk and overgrown, plus the dorsal and lateral arthrodial membranes which connected this somite with the prosoma in front and the mesosoma behind. This double partition then became united into one, the dorsal area of the pregenital somite disappeared, setting free the aorta and the alimentary tract, which were previously confined with the nerve-cord in a narrow canal, and enabling them to rise and take up respectively their original positions in the dorsal and central regions of the body-cavity, cleaving the partition as they rose. Only by entertaining some such conception as this is it possible to hold that the "waist" of the Spiders and

Pedipalpi is the homologue of the "diaphragm" in the Scorpiones and Solifugæ.

This theory of the formation of the diaphragm seems to me scarcely more plausible if the pregenital somite, which was not recognised as such when Bernard wrote, is left out of consideration;¹ and the following quotation shows that I have given no exaggerated rendering of his hypothesis. He says (4), "Between the sixth and seventh segments . . . there is in the Galeodidæ . . . a strong intersegmental constriction. Internally this constriction has given rise to a very striking diaphragm. It forms a very complete wall between the interior of the cephalothorax and that of the abdomen, and is pierced by the dorsal vessel, the alimentary canal, the nerve-cords, and the tracheæ. Close examination shows that the diaphragm is due to a strong indrawing of the intersegmental membrane between the above-mentioned segments, so that it is composed partly of a chitinous infolding and partly of muscle-bands. It is clear that if the opposite two internal faces of such a deep segmental constriction fuse together, they form a diaphragm; if they remain unfused they form a waist. In the Galeodidæ we seem to have an unspecialised arrangement, the intersegmental infolding being fused only in its deeper parts, forming the diaphragm, while the outer parts of the fold remain open, making an approach to a waist."

Such an infolding, in its unspecialised state, must have disturbed the position of the dorsal blood-vessel, forcing it down towards the central axis of the body on to the alimentary canal; but, as a matter of fact, the blood-vessel and alimentary canal show no trace of any disturbance of their primitive positions, the former perforating the diaphragm high up beneath the dorsal integument, the latter traversing its centre, while below, in line, is seen the canal for the nerve-

¹ Bernard has pointed out to me that his figure of the section of the "waist" of a Spider published on pl. xxxiii, fig. 6, of his paper "On the Morphology of the Galeodidæ," shows the presence of a pair of dorso-ventral muscles, and thus confirms the view that the waist is a genuine somite.

cord, to say nothing of the large tracheal apertures on each side. Hence if the diaphragm originated from an integumental infolding it has secondarily encircled the three median organs above mentioned, and is a highly specialised structure. But, as a matter of fact, in the "diaphragm" of *Galeodes* I can find no evidence of such a derivation. It appears to be formed of muscular and connective tissue like that of the Scorpions, and to have had an internal origin quite apart from the integument. The infolding of the integument Bernard speaks of appears to be quite superficial, and to occur only at the periphery of the diaphragm.

Whether this diaphragm has been developed independently of the diaphragm of Scorpions, to which it is similar in its structure and position, or whether the two are to be regarded as a heritage from a common ancestor, are matters of quite another kind. The absence of such an organ in the Pedipalpi, Araneæ, Pseudo-scorpiones, Opiliones, and Acari, coupled with the wide structural divergences between the Scorpions and Solifugæ, points to the independence of the origin of the diaphragm in these two orders in response to similar physiological needs.

NOTE.—I have elsewhere suggested (see 'Ann. Mag. Nat. Hist.,' 1893) that the value of the structural characters of the orders of terrestrial Arachnida may be expressed by grouping them into four divisions of superordinal rank: the first to contain the Scorpions; the second the Pedipalpi and the Araneæ; the third the Solifugæ; the fourth the Pseudo-scorpiones, Opiliones, and Acari. A study of the entosternites confirms this classification in a remarkable and unexpected degree, especially as regards the isolation of the Scorpiones and Solifugæ, and the association of the Araneæ with the Pedipalpi.

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EXPLANATION OF PLATES 13 & 14,

Illustrating Mr. Pocock’s paper, “Studies on the Arachnid Entosternite.”

With the exception of Fig. 1 the figures have been drawn by the author from entosternites dissected and preserved with others, not here figured, in the Natural History Museum, where they are available for examination. In the figures representing the dorsal and ventral views of the entosternite the anterior extremity is uppermost; in those showing the lateral surface the anterior extremity lies to the right. In the case of the so-called entosternite of the Solifugæ, however, the distal or anterior extremity is directed downwards.

LETTERING COMMON TO MOST OF THE FIGURES, AND INDICATING SUGGESTED HOMOLOGIES.

A. P. Anterior process or cornu of right side forming part of the framework of the pharyngeal notch (*Ph. N.*). *1tg.*, *2tg.*, *3tg.*, *4tg.*, *5tg.* Dorsal processes

or muscles representing the dorsal moieties of the tergo-sternal muscles of the somites bearing the first, second, third, fourth, and fifth post-oral appendages. *1st.*, *2st.*, *3st.*, *4st.* Ventral processes representing the ventral moieties of the same muscles. *Cr.* Lateral crest developed mainly to support some of the muscles of the appendages.

PLATE 13.

FIG. 1.—Dorsal view of entosternite of the American *Limulus* (*X. polyphemus*), showing the two pairs of long slender antero-lateral processes (*2tg.* and *3tg.*), the stunted muscle-bearing process (*4tg.*), and the large postero-lateral process (*5tg.*). (After Lankester.)

FIG. 2.—Dorsal view of the entosternite of a Thelyphonid (*Mastigoproctus giganteus*) showing the dorsal processes (*1tg.* to *5tg.*), which are considered to represent the dorsal moieties of the tergo-sternal muscles of the second to the sixth somites of the prosoma; *sa.*¹, supernumerary or additional apophysis, which has perhaps arisen from the fission of the apophysis numbered *2tg.*; *a. b.*, anterior bridge, and *p. b.*, posterior bridge, bounding the large foramen in front and behind; the smaller foramen is shown behind the posterior bridge; *l. b.*, lateral bar, showing perhaps the origin of this portion of the entosternite from a great longitudinal muscle, or from paired subepidermal ventral entochondrites; *P. E.*, posterior plate, with frayed edge indicating the attachment of radiating muscles, the suggested homologue of the dorsal portion of the "diaphragm" in the Scorpions (see Pl. 14, figs. 21, 22); *cr.*, lateral crest.

FIG. 3.—Dorsal view of the entosternite of a Phrynid (*Damon Johnstoni*), showing the duplication of the apophyses numbered *1tg.* and *3tg.*; *cr.*, lateral crest, to which leg-muscles are attached; *1st.*, anterior ventral apophysis, the suggested representative of the sternal moiety of the tergo-sternal muscle of the second segment of the prosoma.

FIG. 4.—Dorsal view of entosternite of one of the Mygalomorphæ (*Ephobopus murinus*), with same lettering as in the last figure, showing the absence of supernumerary apophyses and the presence of a thickened ridge (*p. b.*), the suggested homologue of the posterior bridge in the entosternite of Thelyphonus.

FIG. 5.—Dorsal view of the entosternite of *Stasimopus Schönlandi*, a Mygalomorphous spider of the family Ctenizidæ, showing the presence of two supernumerary apophyses (*sa.*¹ and *sa.*²) and the fusion of the anterior ventral apophyses (*1st.*) to form a neural collar.

FIG. 6.—Dorsal view of entosternite of *Actinopus Wallacei*, a Mygalomorphous spider of the family Actinopodidæ, showing the fusion of the four ventral apophyses to form a subneural arch with perforated walls for the exit

of the appendicular nerves; *1st.*, anterior ventral apophysis; *m.*, median process from the floor of the subneural arch.

FIG. 7.—Dorsal view of entosternite of a young *Liphistius*, sp.?, showing the thick dorsal apophyses (*1tg.* to *4tg.*), the two pairs of supernumerary apophyses (*sa.*¹ and *sa.*²), and the absence of anterior ventral apophyses.

FIG. 8.—Dorsal view of the entosternite of *Nephila femoralis*, an Arachnomorphous spider of the family Argiopidae, with the single pair of supernumerary apophyses (*sa.*¹), and without anterior ventral apophyses, as in *Liphistius*.

FIG. 9.—Lateral view of entosternite of a Thelyphonid (*Mastigoproctus giganteus*) *1st.* and *2st.*, first and second ventral apophyses; other lettering as in Fig. 2.

FIG. 10.—Ventral view of entosternite of *Pachylomerus nidulans*, a Mygalomorphous spider of the family Ctenizidae, showing the persistence of the first (*1st.*) and fourth (*4st.*) ventral apophyses, and the disappearance of the second and third.

FIG. 11.—Lateral view of the same, showing the four dorsal apophyses (*1tg.* to *4tg.*); *p. m.*, posterior median crest.

FIG. 12.—Lateral view of entosternite of *Actinopus Wallacei* (see Fig. 6), showing the four dorsal (*1tg.* to *4tg.*) and four ventral (*1st.* to *4st.*) apophyses, the latter meeting in the middle line beneath the nervous mass, leaving lateral spaces for the exit of nerves; *m.*, median process from subneural arch.

FIG. 13.—Ventral view of entosternite of *Ephebopus murinus* (see Fig. 4), showing the persistence of the four ventral apophyses (*1st.* to *4st.*), with indications of the fifth (*5st.*).

PLATE 14.

FIG. 14.—Lateral view of the entosternite of *Ephebopus murinus*, showing the four dorsal (*1tg.* to *4tg.*) and the four sternal (*1st.* to *4st.*) apophyses. This figure clearly indicates the correspondence between the dorsal and ventral apophyses, which suggests their origin from tergo-sternal muscles (see Pl. 13, figs. 4 and 13).

FIG. 15.—Ventral view of entosternite of *Acanthodon opifex*, a Mygalomorphous spider of the sub-family Idiopinae, showing the persistence of the first, second, and third pairs of apophyses (*1st.* to *3st.*), those of the fourth pair (*4st.*) being rudimentary.

FIG. 16.—Lateral view of the same, showing the duplication of the third and fourth dorsal apophyses, and the absence of the fourth ventral apophysis.

FIG. 17.—Mygalomorphous spider of the genus *Atypus*, dissected from the

dorsal side with entosternite in situ to show the muscles radiating to the post-oral appendages II to VI, the anterior longitudinal muscles which pass from an entosclerite above the rostrum to the extremities of the anterior cornua, and the longitudinal muscles which pass backward into the pregenital somite.

FIG. 18.—Lateral view of entosternite of the same, with the four dorsal (1*tg.* to 4*tg.*) and four ventral (1*st.* to 4*st.*) apophyses, and the tendon running forwards from the first ventral apophysis to the prosternum.

FIG. 19.—Ventral view of the same, showing the arrangement of the four ventral apophyses in a circle round the pharyngeal notch.

FIG. 20.—Entosternite of *Palamnaeus Thorelli*, with the posterior flap or diaphragm removed, showing the fibro-muscular attachment of the anterior cornu to the coxa (*cx.*) of the fourth appendage; *cr.*, lateral processes representing the muscle-bearing crest seen in *Thelyphonus*; 4*tg.*, 5*tg.*, anterior and posterior pair of dorso-ventral muscles, the suggested homologues of the apophyses numbered 4*tg.* and 5*tg.* in the entosternite of *Thelyphonus* (Pl. 13, fig. 2), and representing in all probability the tergo-sternal muscles of the fifth and sixth somites of the prosoma; *V.P.*, median process of subneural arch dividing into a pair of apophyses; *Da.*, median dorsal portion of "body" of entosternite forming the roof of the neural canal.

FIG. 21.—Entosternite of one of the *Vejovidæ* (*Iurus dufourei*), with posterior flap (*P.F.*) or diaphragm attached, to show its correspondence in origin with the lateral crest (*Cr.*), and its median perforations for the gut (*Al.C.*) and aorta (*Aoc.*), between which lies the channel for the lodgment of the aorta, formed by the dorso-ventral muscle of the second pair (5*tg.*) and a strip of connective tissue, which binds the right and left portions of the diaphragm together.

FIG. 22.—Entosternite of *Bothriurus bonariensis*, showing the reduction of the median dorsal portion of the "body," forming the roof of the neural canal, to a narrow transverse bar (*Da.*).

FIG. 23.—Anterior view of entosternite of the same, showing the neural canal (*N.C.*), dorsal arch (*Da.*), and subneural arch (*Sa.*).

FIG. 24.—Entosternite of *Centruroides margaritatus*, with most of the diaphragm removed, showing the lateral compression of the "body" or dorsal arch (*Da.*) of the neural canal, the juxtaposition of the second pair of dorso-ventral muscles (5*tg.*), and the tips of the apophyses of the median process of the subneural arch (*V.P.*).

FIG. 25.—Entosternite of the same, with its dorsal portion removed to show the cut ends of the lateral walls (*La.*) of the neural canal, the floor (*Sa.*) of the latter and the median process terminating in two expanded fan-shaped apophyses (*V.P.*).

FIG. 26.—The so-called entosternite of *Solpuga sagittaria*, cleaned

with caustic potash, to show the continuity of its supporting chitinous strands with those of the prosternal plate (*prs.*), which is wedged in between the coxæ of the appendages of the third pair and its attachment to the sternal membrane (*m.*), between the third and fourth somites of the prosoma; *Is.*, left bar of the entosternite, which expands at the free extremity to form with its fellow of the opposite side a supporting channel (*Al.*) for the alimentary canal.

FIG. 27.—Lower extremity of the two pillars of the entosternite of *Galcodes arabs*, to show this articulation (*a.*) with the prosternal plate (*prs.*), its union with the sternal membrane (*m.*); *cox.*, coxa of appendage of third pair.

On the Morphology of the Cheilostomata.

By

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With Plates 15—18.

THE observations on which the present paper is based were commenced with the examination of a Flustra-like Cheilostome, found at Port Jackson by Mr. T. Whitelegge, who sent it to me for description, believing it to belong to a new genus. Although exhibiting several remarkable features, I think it may be placed in *Euthyris*, Hincks, and I propose for it the name *E. clathrata*, in allusion to the bars which support its "frontal wall." The species possesses a large "compensation-sac," a name which is due to Jullien (1888, 1), although the structure had to some extent been described by Busk and Waters. Jullien's results have usually been discredited by later writers. The study of a number of Cheilostomatous genera has, however, not only led me to confirm the accuracy of Jullien's statements, but has resulted in various conclusions which I believe to be of importance for the proper understanding of the Cheilostomata. A summary of my principal results has been communicated to the Cambridge Philosophical Society (1901).

The present paper is divided into the following parts:

- I. Methods employed.
- II. List of the species specially studied.

- III. Description of *Euthyris clathrata*, n. sp., of *E. obtecta*, Hincks, and of *Euthyroides episcopalis*, n. gen.
- IV. The morphology of the compensation-sac and of the operculum.
 - A. Flustrina.
 - B. Cribrilinidæ.
 - C. Lepralioid genera.
 - D. Microporelloid genera.
 - E. Microporoid genera.
- V. The primary zoœcium or ancestrula.
- VI. Classification of the Cheilostomata.
- VII. Summary.
- VIII. Literature.
- IX. Explanation of Plates.

I. METHODS EMPLOYED.

The choice of species for investigation has been primarily dependent on the material available for the purpose in the collection of the University Museum of Zoology at Cambridge. Spirit material has almost exclusively been used, and I have in the main selected species in which the calcareous matter was not developed to so great an extent as to destroy the transparency of the object. The growing edges of healthy colonies have furnished the most satisfactory results. The material was in almost all cases stained, without decalcification, in diluted borax carmine for a prolonged period (five to seven days, or even more). After being placed in absolute alcohol containing picric acid, the fragments were mounted whole in Canada balsam.

All my more recent preparations have been mounted by a method to which my attention was called by Mr. H. D. Geldart, of Norwich. This consists in transferring the specimens directly from absolute alcohol to a solution of dried Canada balsam in absolute alcohol. In preparing this solution, the milky mixture which is at first produced becomes a

complete solution in the course of a few days, particularly if the bottle be left on the top of a water-bath at about 60° C. The cloudiness which appears on first mounting a preparation soon disappears from a slide left on the water-bath. This method cannot be too strongly recommended for certain Polyzoa, particularly for the more delicate Ctenostome genera, which are distorted almost beyond recognition by the use of oil of cloves.

In the case of some of the more densely calcified species I have found great advantages in the use of the method recommended in my paper on *Steganoporella* (1900, p. 240) of removing the basal wall, by means of a scalpel, from stained colonies (not decalcified) embedded in paraffin. I have also made use of thin slices, cut by hand, of uncalcified material embedded in paraffin. I am convinced of the great importance of studying the Cheilostomata in uncalcified Canada balsam preparations. Most of my slides have been examined with a binocular microscope and a quarter-inch objective.

II. LIST OF THE SPECIES SPECIALLY STUDIED.

1. *Euthyris clathrata*, n. sp. . . . Port Jackson.
2. „ *obtecta*, Hincks . . . Torres Straits.
3. *Euthyroides episcopalis*, Busk
(n. gen.) Victoria.
4. *Flustra pisciformis*, Busk . . . Bass's Strait (Challenger Coll.).
5. „ *papyrea*, Pall. Naples.
6. „ *cribriformis*, Busk . . . Singapore.
7. *Farciminaria hexagona*, Busk . . Amboina (Challenger Coll.).
8. *Dimetopia spicata*, Busk. . . . Victoria.
9. *Bicellaria grandis*, Busk, var. *producta*, MacGillivray Victoria.
10. *Bugula neritina*, L. Naples.
11. *Membraniporella nitida*, Johnst. . S. Devon.
12. *Cribrilina philomela*, Busk . . Marion Is. (Challenger Coll.).
13. „ *radiata*, Moll Naples.
14. *Umbonula verrucosa*, Esper . . S. Devon.
15. „ *pavonella*, Alder . . . North Sea.
16. *Lepralia pallasiana*, Moll . . . Naples.

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|-----|--|---|---|---|
| 17. | <i>Lepralia dorsiporosa</i> , Busk | . | . | Torres Straits. |
| 18. | " <i>sincera</i> , Smitt | . | . | Davis Straits. |
| 19. | " <i>haddonii</i> , n. sp. | . | . | Torres Straits. |
| 20. | <i>Schizoporella linearis</i> , Hassall | . | . | Naples. |
| 21. | " <i>sanguinea</i> , Norm. | . | . | Naples. |
| 22. | " <i>australis</i> , Haswell | . | . | Torres Straits. |
| 23. | <i>Urceolipora nana</i> , MacGill. (= <i>Calymmophora lucida</i> , Busk) | . | . | Victoria. |
| 24. | <i>Smittia trispinosa</i> , Johnst., var. <i>arborea</i> , Levins. | . | . | Greenland. |
| 25. | " <i>reticulata</i> , J. MacGillivray | . | . | Naples. |
| 26. | <i>Catenaria lafontii</i> , Aud. | . | . | Naples. |
| 27. | <i>Vittaticella cornuta</i> , Busk | . | . | Victoria. |
| 28. | <i>Catenicella alata</i> , Wyv. Thoms. | . | . | Victoria. |
| 29. | " <i>plagiostoma</i> , Busk, var. <i>setigera</i> , MacGill. | . | . | Victoria. |
| 30. | " <i>hastata</i> , Busk | . | . | Victoria (Challenger Coll.). |
| 31. | " <i>lorica</i> , Busk | . | . | Victoria. |
| 32. | " <i>wilsoni</i> , MacGillivray | . | . | Victoria. |
| 33. | <i>Calwellia gracilis</i> , Maplestone | . | . | Victoria. |
| 34. | " (<i>Onchopora</i>) <i>sinclairii</i> , Busk | . | . | S. of Kerguelen Is. (Challenger Coll., Stat. 153). |
| 35. | " (<i>Urceolipora</i>) <i>dentata</i> , MacGillivray | . | . | Victoria. |
| 36. | <i>Ichthyaria oculata</i> , Busk | . | . | S.E. of Buenos Aires (Challenger Coll., Stat. 320). |
| 37. | <i>Onchoporella bombycina</i> , Busk (not Ell. and Sol.) | . | . | New Zealand. |
| 38. | <i>Microporella malusii</i> , Aud. | . | . | Naples. |
| 39. | " <i>ciliata</i> , Pall. | . | . | Naples. |
| 40. | <i>Micropora</i> , sp. | . | . | Torres Straits. |
| 41. | <i>Steganoporella alveolata</i> , Harmer | . | . | Torres Straits. |

III. *Euthyris*, Hincks, and *Euthyroides*, n. gen.

Euthyris clathrata, n. sp. Pl. 16, figs. 18—31.

Zoarium Flustrine in habit, of stiff, corneous texture, composed of narrow, parallel-sided, frequently bifurcating branches, with truncated ends. Zoœcia opening on one surface only, the orifices arranged with great regularity in

oblique rows passing entirely across the branch in two intersecting directions. Orifices apparently connected by a continuous, brown, transparent epitheca, a short distance below which the frontal surface of each zoöcium is strengthened by a system of irregular calcareous bars, which tend to radiate from a point in the middle of the base-line of the operculum towards the proximal and lateral sides of the zoöcium. Basal side of the branch similarly covered by an epitheca, which each zoöcium reaches along a longitudinal line narrower and shorter than itself. Opercula large, dimorphic, the ordinary form about as long as broad (250 to 270 μ), the others with a broader base (290 to 320 μ). Both kinds of opercula are strengthened by a conspicuous Ω -shaped sclerite. The distal margin of the vestibule is provided with a chitinous lip, which is overlapped during retraction by the large lateral flanges of the operculum. Ovicells not found, and probably absent.

The material was discovered by Mr. T. Whitelegge under rock ledges, at low-tide line, Watson's Bay, Port Jackson, and in Middle Harbour, Port Jackson. Although part was in spirit, its condition was not sufficiently good to make a complete anatomical investigation possible.

The genus *Euthyris* was founded by Hincks¹ for a new species, *E. obtecta*, from North Australia. The generic name, introduced "to suggest the idea of higher structure" in the operculum, is particularly appropriate to *E. clathrata*, in which the operculum is specially complicated. The two

¹ 'Ann. Mag. Nat. Hist.' (5), x, 1882, p. 164. In 1871 Quenstedt ('Petrefaktenkunde Deutschlands,' Abth. I, Bd. ii, p. 442), in discussing the structure of a Brachiopod, used the following words:—"Man könnte sie daher wohl *Euthyris* aber nicht *Athyris* nennen." I have not been able to ascertain that Quenstedt made any further use of the word *Euthyris*, and he does not even refer the species he is discussing to that genus. It appears, therefore, that he was not in reality proposing a new genus (and he certainly did not define it), but was merely making a verbal criticism of the name *Athyris*. Although *Euthyris* (Quenstedt) is mentioned by Zittel in his well-known 'Handbuch d. Palæont.' (i, p. 684) as a synonym of *Spirigera*, it does not seem to me that it has any valid claim to recognition.

species agree in their habit, in their dimorphic opercula (probably associated with the absence of ovicells), and in the highly developed chitinous epitheca which overspreads the entire zoarium. *E. clathrata* differs from *E. obtecta* in having its frontal calcareous wall composed of irregular bars, instead of a simple, perforated, calcareous film, and in the fact that there is no large space between the frontal epitheca and the calcareous walls.

I feel doubtful whether *Euthyris woosteri*, MacGillivray,¹ is rightly referred to this genus; but, on the other hand, *Carbasea moseleyi*, Busk (1884, p. 56), perhaps belongs to it.

The largest colony of *E. clathrata* measures about 18·5 cm. or 7½ inches in length. The branches are 2 to 4 mm. wide, averaging about 3 mm. near their free ends, but lessening towards the base of the colony, which appears to have been attached by a narrow base without rootlets. A branch 4 mm. wide has about thirteen orifices in each oblique row. The colour is brown in the older parts, yellowish near the ends of the branches. The zoarium frequently bifurcates, showing some tendency to form a unilateral cyme. The terminal divisions (Pl. 16, fig. 18) may reach a length of 3·5 cm. without bifurcating, but the ordinary length of the divisions is not more than 1·5 cm. The frontal surface is somewhat convex, the opposite surface flatter. The calcareous walls of the zoecia are arranged as follows:—The lateral and terminal walls are everywhere complete, and are perforated by numerous pores. At the proximal end (fig. 22) the two lateral walls pass continuously into one another in a regular curve, which forms the base of the zoecium, and is placed some distance within the basal epitheca (fig. 26, *b. ep.*). At the distal end the lateral walls approach one another basally, and are separately inserted into the basal epitheca, forming a linear mark (figs. 22, 27) constantly shorter than the zoecium, but varying in length; this is connected with the similar part of the next zoecium by a chitinous “mesen-

¹ ‘Proc. R. Soc. Vict.’ (N. S.), iii, 1891, p. 77.

tery" (fig. 22, *m.*). The effect of the arrangement indicated in fig. 27 is to keep the epitheca stretched as a flat membrane at some distance from the basal calcareous walls of the zoëcia. The cavity beneath the epitheca is divided into a series of parallel longitudinal spaces by the parts of the zoëcia above described. The linear figure formed by the insertion of the zoëcium into the basal epitheca is in some cases bifurcated proximally. On the frontal surface (figs. 20, 21) each orifice is surrounded by a somewhat irregular ring of calcareous matter, from each side of which is given off a strong condyle (fig. 21, *cond.*) or "denticle," the two condyles forming the hinge of the operculum. The frontal surface is strengthened by a highly variable arrangement of calcareous bars, the general position of which is shown in fig. 20. The bars are in the main flattened, their flat surfaces being parallel with the surface of the branch, but in curving down into the lateral walls they usually give off vertical flanges from their free surface, and these form bridges across the depressed intervals between two zoëcia, joining similar flanges in the adjacent zoëcia. At the free end of the branch the proximal parts of the bars are first formed, and they grow in a distal direction beneath the epitheca.

Along the lateral margin of the branch runs a tube (fig. 20, *m. c.*)¹ which has usually been described as a "chitinous fibre" in other forms of Flustrine habit. This is merely a part of the branch which is not divided into zoëcia, and calcareous bars (*c. b.*) extend from the marginal zoëcia nearly to the outer edge of its free surface; it contains, moreover, strands of funicular tissue which pass across its lumen. This space runs as a continuous tube along the whole margin of the branch, and it communicates with the cavity which lies between the backs of the marginal zoëcia and the basal epitheca.

E. clathrata, like *E. obtecta*, is characterised by the dimorphism of its opercula. This is shown in figs. 20, 21, representing the ordinary type ("A") and the second form

¹ Cf. Waters, 1896, p. 291.

("B"). The zoëcia to which the two kinds of opercula respectively belong also show some dimorphism. In the A-zoëcia the condyles for the articulation of the operculum are long recurved teeth (fig. 21), while in the B-zoëcia they are short tubercles. The distal calcareous wall of the A-zoëcium is at the same time the proximal wall of the next zoëcium in the same longitudinal line. In the B-zoëcium this is not the case. The distal zoëcium has a proximal wall (*p. w.*) of its own, from which some of its calcareous bars may spring, and this is much thinner than the distal wall (*d. w.*) of the proximal zoëcium, from which it is separated by a narrow crescentic space, passing about half round the operculum of the proximal zoëcium. This suggests that the B-zoëcia possess a vestigial ovicell. The condition of my specimens unfortunately prevents me from ascertaining whether the production of ovaries is limited to B-zoëcia. In two cases counted at random, about three A-zoëcia occurred for every B-zoëcium, no regular arrangement of the two kinds being apparent. The additional breadth of the B-operculum is correlated with a slightly increased transverse diameter of the zoëcium itself, immediately on the proximal side of the operculum. This makes an appreciable difference in the capacity of the zoëcium, a fact which is in favour of the view that the B-zoëcia are female. Similar differences in the opercula are commonly met with in other Cheilostomes, in which the operculum of an ovicell-bearing zoëcium may be wider than that of the ordinary zoëcia.

Two features in *E. clathrata* demand especial attention, namely, the compensation-sac and the operculum. For the discovery of the compensation-sac Jullien (1888, 1) is entitled to full credit,¹ although his results have been re-

¹ See my preliminary paper (1901) on this subject. Jullien's accounts of the compensation-sac were very short, and his figures were not adequate. He nowhere brings out the importance of the sac in the discussion of the morphology of the Cheilostomata. So far as I know, he mentions its parietal muscles in only one place. This is in the explanation of a figure of *Cribrella figuraris* (1888, ii, p. 272), in which he uses words which seem to

ceived with scepticism, which proves to have been unjustified.

The COMPENSATION-SAC of this species is a very large cavity which underlies practically the whole of the frontal surface of the zoœcium. In longitudinal section (fig. 26, *c. s.*) it is seen to be perfectly distinct from the body-cavity, from which it is separated by a delicate membrane, constituting the floor of the sac. This membrane passes continuously into the base-line of the operculum, immediately proximal to which the sac opens to the exterior. When the operculum is closed the aperture of the sac is a virtual transverse slit. In the case of ordinary Escharine forms this aperture is so little apparent that it has commonly been supposed that the base-line of the operculum actually articulates with the adjacent part of the calcareous wall—a state of affairs which is often by no means the case. The roof of the compensation-sac is protected by the calcareous bars (*f. b.*), and the frontal wall of the zoœcium is bevelled off on the proximal side of its aperture, in the characteristic way shown in fig. 26. The purpose of this arrangement is obvious. The sharp edge of the frontal wall of the zoœcium is in contact with the base-line of the operculum when the latter is closed. When the operculum is open (fig. 24) the plane of its free surface becomes parallel with that of the bevelled edge of the frontal wall, so that water can have unobstructed access to the sac. As supposed by Jullien, there can be little doubt that the entry of water into the sac renders the protrusion of the polypide possible, but it almost necessarily follows that the constant change of the water in the sac makes this structure an important organ of respiration.

In this species I have been able to obtain only unsatisfactory evidence, in consequence of the state of its preserva-

indicate that he had appreciated the morphological importance of his discovery. The words are as follows :—"Muscles rétracteurs de l'abdomen (fibres musculaires pariétales des auteurs). Elles rétractent en réalité la paroi abdominale, mise en évidence par la découverte de la chambre à eau de compensation ou chambre compensatrice."

tion, with regard to the existence of the "parietal muscles" which elsewhere dilate the compensation-sac; but the analogy of other Cheilostomes leaves little doubt as to their presence.

The OPERCULUM is a very remarkable piece of mechanism, which forms a most efficient means of protecting the entrance to the tentacle-sheath. Most of the existing descriptions of Cheilostomatous opercula take account of the appearance of the outer surface only of this structure—a very inadequate way of arriving at its real relations. Although *E. clathrata* has an operculum which, judged by the descriptions available for comparison, would appear to be unusually complex, it is in the highest degree probable that a renewed examination of other Cheilostomes will show that it is by no means unique in this particular.

The first part of the introvert which leads to the mouth of the polypide is constituted by the "diaphragm" or "vestibule" (fig. 26, *vest.*), which is a muscular invagination projecting into the tentacle-sheath, and communicating with it by a central aperture.¹ The way in which the vestibule opens and closes has been aptly compared with the action of a clasp-purse. The structures which protect the vestibule of *E. clathrata* have a superficial resemblance to the skull of a turtle (*Chelone*), the skull with the upper jaw being represented by the operculum, and the lower jaw by a chitinous lower lip (figs. 23—27, *lb.*), which I propose to term the "labium." The labium was described by Hincks² in a form from the Queen Charlotte Islands, named by him *Lepralia bilabiata*, in allusion to the existence of this structure. It is probable that it will hereafter be found in numerous Cheilostomata.

The labium can be clearly seen in those zoöcia of the dry colony in which the operculum is open (fig. 19). Between it and the edge of the downwardly projecting flange (*fl.*) of the

¹ See, for a description of this structure, Nitsche (1871, p. 432), Jullien (1888, 4, p. 38), and Calvet (1900, pp. 180, 201).

² 'Ann. Mag. Nat. Hist.' (5), xiii, 1884, p. 49. The labium is the "upper lip" of Hincks's description.

operculum is seen the entrance to the vestibule, while the aperture of the compensation-sac is situated between the base of the operculum and the calcareous frontal wall of the zoëcium.

The relations of the same parts are explained by the thick longitudinal section shown in fig. 26, in which the operculum is very nearly closed. By deep focussing can be seen the calcareous condyle (*cond.*) which constitutes the hinge. The divaricator muscles (*div.*) of the operculum are paired; each originates from one of the lateral walls of the zoëcium, and passes obliquely towards its frontal surface, crossing the condyle on its proximal side, to reach its insertion into the basal sclerite of the operculum. The contraction of these muscles will obviously have the double effect of opening the vestibule and of opening the compensation-sac. The operculum is prolonged laterally into a very large triangular flange (*fl.*), whose plane is at right angles to that of its free surface (see also figs. 24, 25, 27). The occlusor muscles (*occl.*) are similarly paired, each originating from the lateral wall of the zoëcium at a deeper level than the divaricator, and passing obliquely across the base of that muscle to reach its insertion, on the distal side of the condyle, into the tip of the triangular flange. Fig. 25 shows that the insertion is by means of a broad tendon. Since the labium articulates with the operculum in this region, the effect of the contraction of the occlusor muscles will be, not only to close the operculum itself, but also to retract the labium from the position shown in fig. 24 to that shown in fig. 25. In the closed condition, the labium lies just inside the vertical flange of the operculum.

The lateral flanges are not really independent structures, but they pass into one another in a continuous curve round the distal side of the operculum, their free border, continuing the comparison with the turtle's skull, constituting the biting edge of the upper jaw. In longitudinal section (fig. 26) the upper jaw appears to be strengthened by a strong buttress (*buttr.*) which passes from the free surface of the operculum.

This is a part of the Ω -shaped figure which is seen in a surface view (fig. 21) of the operculum, an appearance which results from the fact that the thickness of the chitin is not everywhere the same. The whole of the region included within the two limbs of the Ω is much thickened (figs. 26, 27), while the rest of the free surface, as far as the edge, is composed merely of thin cuticle (fig. 29). From the median thickening a curved chitinous buttress diverges (figs. 28—31) on each side, passing down into that part of the body-cavity which immediately underlies the free surface of the operculum. In young zoëcia, the space between the buttress and the outer lamella of the operculum contains a conspicuous epithelium, which secretes the cuticular substance of which the operculum is composed. Remains of this cellular material may be seen, in the same position, in the adult operculum.

The greater part of the thickness of the operculum is constituted by a superficial layer of cuticle which readily stains yellow with picric acid. The whole of the deeper surface of this layer (and therefore both sides of the strengthening buttress which depends from the free surface), is lined by a much thinner layer of chitin (not indicated in the figures), which takes a red colour in sections stained with borax carmine and picric acid. A two-layered chitinous cuticle has been described in certain Gymnolæmata by Calvet (1900, p. 164).

The buttress of the operculum is crescentic in shape, as seen from above (fig. 21, *buttr.*). Thus, if the thin lateral parts of the outer lamella of the operculum were removed, there would be exposed a crescentic cavity, the limbs of the crescent being directed towards the proximal side of the zoëcium, the floor of the cavity being constituted by the slightly concave upper surface of the buttresses. Distally the buttress meets and fuses with the vertical flange of the operculum (fig. 26). Laterally the fusion is incomplete distally (figs. 27, 28), so that the cavity above the buttress is here continuous with the general body-cavity. In the

proximal half of the operculum the buttresses again meet the lateral flanges (figs. 29—31).

The distal half of an operculum is shown in fig. 27, which represents a transverse slice of a specimen with its calcareous parts, cut freehand¹ after embedding in paraffin. By focussing deeply are seen the labium (*lb.*) and the curved slit between it and the edge of the operculum. Into the ends of the lateral flanges of the operculum, and at the same time into the two sides of the base of the labium, are inserted the occlusor muscles (*occl.*). By focussing less deeply the free edges of the lateral buttresses are seen; while the cut surface passes through the region of the condyles (*cond.*). Fig. 26 shows that the operculum works on its condyles as a lever of the first order, the occlusors (*occl.*) and the divaricators (*div.*) passing on opposite sides of the fulcrum.

Fig. 28 shows a transverse section of the distal part of the operculum where the buttresses have a free edge. Fig. 29 is through the hinge-line. The vertical flange is still deep (cf. fig. 26), and is here strengthened by the buttresses, which have completely fused with it. On the proximal side of the condyles the depth of the flange rapidly diminishes. In the entire operculum a small circular mark (fig. 21, *w*) appears on each side near the proximal end of the Ω -shaped figure. In transverse section (fig. 30) this is seen to be due to the fact that at this point the buttresses leave the vertical flanges and are inserted into the free wall of the operculum, so that at this point the body-cavity is separated from the outside by a single layer of chitin. The small tube thus formed often stains deeply in carmine preparations. I am unable to explain the meaning of this arrangement. The tube does not communicate with the space above the buttresses. On its proximal side the operculum becomes very shallow, as indicated in fig. 31, a section just on the distal side of the basal sclerite. This forms, with the median thickening, a horizontal \perp -shaped figure, the outline of which

¹ The hard nature of the chitin makes it difficult to prepare microtome sections of the opercula.

gives rise to the Ω -shaped mark seen in an external view of the operculum (fig. 21).

In decalcified sections the calcareous part of the condyle is seen to be surrounded by a strong layer of chitin. In longitudinal sections this has the appearance indicated in figs. 25, 26 (*cond.*), the chitin being developed especially on the proximal side of the condyle. Fig. 24, which represents an open operculum, shows that this chitinous investment is prolonged into the sharp edge (*scl.*) which surrounds the cavity in which the operculum lies (see also figs. 27, 29). This edge is continuous with a layer of chitin forming the outer wall of the cavity (figs. 28—31); and this, in its turn, becomes continuous with the deep end of the vertical flange of the operculum. The flange is strongly bent inwards so as to lie along the surface of the condyle.

I think there is great probability that these arrangements are in the nature of a spring, and that if the partially opened operculum shown in fig. 29 be imagined to be closed by the occlusor muscles, the shape of the lateral flanges and of the buttresses will be somewhat distorted by the surfaces of the condyles. The elasticity of the operculum would thus tend to commence the opening of the orifice and of the compensation-sac. This supposed elasticity probably gives a reason for the free ends of the buttresses in the distal part of the operculum, an arrangement which would facilitate the slight alterations in the vertical flanges during the movements of the operculum.

The whole apparatus, consisting of the operculum, the labium, and the chitinous cutting edge which surrounds the cavity containing the operculum, is obviously one of remarkable perfection, and is well adapted to defend the entrance to the tentacle-sheath.

The most external part of the vestibule is lined by a distinct layer of cuticle (fig. 26), which is continuous with the cuticle of the operculum and with that of the labium. The actual "biting" surface of the jaws is constituted by a thickened part of the cuticle, as shown in the same figure.

That of the operculum is so close to the insertion of the buttress into the vertical distal wall as to receive support from the buttress. That of the "lower jaw" is continuous with the main thickening of the labium, which is the deeper wall, as shown in fig. 26.

I have few observations on other points in the structure of this species. In the material at my disposal a certain amount of disintegration appears to have taken place before the specimens were preserved. The number of tentacles is, however, about twenty-two.

Euthyris obtecta, Hincks,¹ Pl. 16, figs. 32—37.

The B-zoœcia are much larger than the A-zoœcia, the ratio in volume being probably at least 2 : 1 (figs. 32, 35). The A-opercula are of much the same shape as in *E. clathrata*, while the B-opercula are relatively much broader. The condyles are weaker than in *E. clathrata*, and are situated nearer the proximal border of the operculum. But what most strikingly differentiates the two species is the character of the calcification. The calcareous wall is everywhere complete in *E. obtecta*, the basal wall being uniformly rounded along its whole extent. Both kinds of zoœcia are flask-shaped (figs. 34, 36), the opening of the neck of the flask being filled by the operculum. A continuous flat epitheca overspreads the entire zoarium, being stretched out in the plane of the opercula on the frontal surface of the branch, and at a considerable distance from the zoœcia on the basal surface (fig. 34, *ep.*, *b. ep.*). The space beneath the epitheca (*ep. c.*) is thus extremely large, and that of the frontal surface is continuous with that of the opposite side by means of the undivided space which runs along each edge of the frond (figs. 32, 35, *m. c.*).

On the frontal surface the epitheca is supported in the main by the edges of the orifices of the zoœcia, but in addition by a few calcareous tubercles (fig. 34, *calc. p.*), which arise irregularly from various points of the surface of

¹ 'Ann. Mag. Nat. Hist.' (5), x, p. 164; (6), xi, p. 177.

the calcareous wall (fig. 32), and pass vertically to the epitheca. Basally these tubercles are longer and are rather more regularly arranged. Their arrangement is seen in figs. 34—36, from which it is apparent that they serve to keep the epitheca stretched out at a considerable distance from the calcareous part of the zoëcium. In my preliminary note (1901, p. 16) I have pointed out that there is evidence that many Cretaceous Cheilostomes were provided with a basal epitheca, as is indicated by the presence of calcareous papillæ resembling those of *E. obtecta*. It may perhaps be suggested that a function of the epitheca is to protect the calcareous walls from the attacks of boring organisms (e. g. the Infusorian *Folliculina*) which infest many calcareous Polyzoa.

The calcified wall of *E. obtecta* shows no trace of the bar-like arrangement so characteristic of *E. clathrata*. It is, on the contrary, a continuous calcareous film, traversed by pores. As in the other species, these pores partly form communication-pores traversing the partition-walls between zoëcia, and they are partly in relation with the space beneath the epitheca. It is hardly necessary to point out that in neither case are they open pores, although the calcareous matter is deficient in these regions. The portions of the vertical walls which are actual partition-walls are of limited extent (fig. 36), so that but few of the pores are communication-pores (*c. p.*) between zoëcia. I have seen no evidence that the number of these is restricted to eight, as stated by Waters (1896, p. 282). The majority lead to the spaces beneath the epitheca, particularly to that on the basal side of the frond (figs. 35, 36). They are less numerous on the frontal side (fig. 36).

The marginal part of the frond may be strengthened by a system of bars (fig. 37) which recalls those already described in the zoëcia of *E. clathrata*. This system makes its appearance first in the angle between the two lobes of a bifurcation, and begins as a set of regular calcareous bars, running near the frontal surface, and originating from the

outer sides of the marginal zoëcia. Each bar is at first narrow, but soon dilates into a thickened, tuberculated, rounded head, the heads being closely apposed to form a continuous calcareous border. From the border a longitudinal vertical ridge stands up at right angles to the frontal epitheca, which it meets. A similar ridge (*l*) connects the border with the basal epitheca. In older branches each bar may be dilated at two points, and a second longitudinal ridge may be formed on each surface (fig. 37, *l*, *l*).

The marginal thickening,¹ thus constituted of a series of calcareous thickenings, gives flexibility as well as strength to the margin of the frond. There can be little doubt that the space beneath the epitheca is a kind of colonial body-cavity. The marginal bars of *E. obtecta* can thus be regarded as directly comparable with the zoëcial bars of *E. clathrata*. From the irregularity and variability of the bars in the latter I am inclined to regard this as a species in which the calcification has been reduced from a condition like that in *E. obtecta*.

The general arrangement of the viscera in *E. obtecta* is shown in fig. 34. The operculum has conspicuous lateral flanges, and there is a delicate labium (*lb.*). The ocluser muscles (*occl.*) are inserted into the apices of the lateral flanges. Some of the fibres of the divaricator muscles (*div.*) appear to reach the base-line of the operculum, but some are probably inserted into the adjacent part of the floor of the compensation-sac (*c. s.*). This structure is very large, and in an adult zoëcium it underlies the whole of the frontal surface. Fig. 33 shows its appearance in a B-zoëcium in back view. The greater part of the basal wall (*b. w.*) of the zoëcium and part of the polypide have been removed. The tentacle sheath depresses the sac medianly, but the sac bulges out on each side into a strongly convex lateral lobe. Each lobe thus formed is rounded off distally, but by deep focussing the two lobes can be seen to unite on the far side of the tentacle sheath into a single cavity, which can be traced to

¹ A similar thickening is well known in certain other flexible Cheilostomes. See, for example, Levinsen's account (1891, p. 274) of *Flustra carbasea*.

the proximal border of the operculum. The divaricator muscles are seen at *div.*; *occl.* indicates the position of the origin of one of the ocluser muscles, while a pair of parieto-vaginal muscles are seen at *p. v. m.* Arising from the sides of the zoëcium are a series of delicate parietal muscles (*p. m.*). There is a tendency for these to be arranged in a grouped manner. They can be traced along the basal surface of the compensation-sac, into which each is inserted.

A polypide and a compensation-sac occur in both forms of zoëcia. Reproductive organs are, unfortunately, absent, so that it is not possible to ascertain whether the dimorphism has any relation to reproduction.

The compensation-sac develops in what I shall term the Lepralioid manner; that is to say, as an invagination formed at the base of the operculum after the calcification of the front wall has been completed. Some of the details of this process are described in the account of the next species.

Euthyroides, n. gen.—I suggest this term for *Carbasea episcopalis*, Busk,¹ a form placed by Hincks² in the genus *Euthyris*. The diagnosis of the genus may be given as follows:

Zoarium of *Flustra*-like habit, bordered along each edge by a tube, interrupted at intervals, which represents a part of the body-cavity not divided into zoëcia, but without other spaces beneath the epitheca. Frontal wall more or less calcareous, covering a well-developed compensation-sac. Communication-pores large, typically four on each side. Ovicells large, external, with a wall composed of two calcareous layers.

The genus differs from *Euthyris* in the absence of spaces beneath the epitheca and in the presence of large external ovicells, and of a very different type of communication-pore.

The zoëcia of *E. episcopalis* (Pl. 15, figs. 13—17) are extremely elongated. Their vertical and basal walls are calcified, but there is so little calcareous matter in the frontal

¹ Busk (1852), p. 52; MacGillivray, 'Prod. Zool. Vict.,' I, Dec. v, 1880, p. 28.

² 'Ann. Mag. Nat. Hist.' (5), x, p. 164.

wall that this part may not entirely retain its shape in drying.

Large, oval communication-pores, or rosette-plates, occur in the lateral walls, at about the middle of the interval between the frontal and basal surfaces (fig. 14, *c. p.*). Each zoecium is normally bounded by two zoecia on each side, and it usually communicates with each of its four lateral neighbours by means of two (rarely three) rosette-plates. In the terminal partition-walls, there is a horizontal row of small pores in place of definite rosette-plates. The vertical walls have no other pores.

The transparency of this species makes it a favourable one for the study of the compensation-sac. At the growing ends of the branches the frontal surface of the zoecium is at first an uncalcified membrane, in which calcification begins at the proximal end and gradually extends distally. The outline of the operculum becomes apparent before calcification invades its immediate neighbourhood. Shortly before the edge of the calcified frontal wall (fig. 13, *a*) reaches the region of the future operculum, the part of the uncalcified membrane immediately proximal to the opercular base-line shows a special accumulation of nuclei (*c. s.*), towards which a number of muscle-fibres radiate through the body-cavity from both lateral walls. When the calcification has advanced so far as to mark out the future orifice, two lateral calcified processes and a median tongue-like structure begin to grow up just proximal to the operculum (fig. 14, *l. p.*, *tg.*). The nuclei are arranged in a more definite mass along the proximal margin of the orifice, to which the lumen of the tentacle-sheath (*t. s.*) now extends. Still later, the two calcareous processes meet, although the suture between them is persistent. There is thus left, between them and the calcareous tongue, a crescentic pore (fig. 15), the concavity of which is directed proximally. This is an aperture of the compensation-sac, which, however, opens to the exterior at the proximal edge of the operculum as well, an arrangement which is most obvious in the fertile zoecia. The oclusor muscles of the

operculum are large, and originate from the lateral parts of the basal wall, close to the distal end of the zoëcium. The divaricator muscles originate from the lateral walls of the zoëcium, in the same neighbourhood.

The zoarium is bounded by a tubular cavity, which is, however, not continuous, as in the preceding species. The cavity is a direct prolongation of one of the longitudinal lines of zoëcia, and may be compared with what Smitt calls a "Samknopp" (1865, p. 6). After extending a length of perhaps five ordinary zoëcia it ceases abruptly, and a new marginal tube is formed as a prolongation of the series of zoëcia next internal to it. The lateral tubes are connected with the adjoining zoëcia by communication-pores.

The ovicell (fig. 16) of this species is well known from Busk's description. It is very prominent, and is provided with a median longitudinal keel, on each side of which is a large elongated fenestra (*f.*). This is simply a membranous deficiency in the outer wall of the ovicell, which is composed of two calcareous layers. The fertile zoëcium is distinguished from the others by a peculiarity in its calcareous frontal wall. Instead of having a single pair of prominences on the proximal side of the operculum, it has several such prominences (figs. 16, 17), the arrangement of which is somewhat variable. There are either several tubular calcareous bars radiating towards a point in the middle of the zoëcium (fig. 16), or a short series of bars disposed more definitely in pairs (fig. 17). The latter arrangement seems to occur typically in those fertile zoëcia which have an ovicell on their proximal side, and the former in a fertile zoëcium which is the first of a longitudinal series.

The resemblance of these bars to the frontal bars¹ of a

¹ I suggest the term "frontal membrane" for the membranous body-wall which is stretched over the "aperture" of a *Membranipora* (fig. 43). The "frontal bars," or "costules" of some authors, are the bars which grow over this membrane in a *Cribrilina* (figs. 8, 44); while I propose the term "frontal shield" for the calcareous part of the frontal surface of a *Cheilostome*. The frontal shield is probably not homologous in all *Cheilostomes*, but the term may be used as a purely descriptive one.

Cribrilina is very striking. They arch over a greatly reduced frontal membrane, into which parietal muscles are inserted in the immature condition of the zoëcium (fig. 17). The bars are calcareous tubes, opening by a foramen into the body-cavity just external to the frontal membrane, and each bar has a minute membranous fenestra near its tip. After the complete formation of the bars there is found (in the fertile zoëcium) a stage in the development of the compensation-sac precisely like that shown in fig. 15 in a zoëcium without an ovicell, and it can now be seen that the operculum is continuous with the floor of the compensation-sac, a wide opening into which is left between the operculum and the first pair of bars. In the mature fertile zoëcium the compensation-sac extends under the greater part of the frontal shield, as in the ordinary zoëcia.

There is little difference between the relations of the frontal bars in the fertile zoëcia of *E. episcopalis* and that found in certain species of *Cribrilina* (e. g. *C. figularis*), in which the frontal membrane surrounded by the bars is of reduced extent.

In the immature ovicell of *E. episcopalis* (fig. 17) the inner calcareous wall is a concave plate (*i. w.*) lying on the surface of the zoëcium next distal to that to which the ovicell belongs. The outer calcareous layer (*o. w.*) rises up concentrically outside it, and between the two is a mass of living tissue. It is impossible not to be struck by the resemblance between the development of the ovicell and that of the frontal bars. The ovicell may be compared with two greatly expanded bars, composed, like the others, of two layers of calcareous matter surrounding living tissue.

The median keel of the mature ovicell represents the line along which these bars meet, and corresponds, I believe, with a complete septum between their cavities. It may thus be suggested that the ovicell is formed by the fusion of a pair of greatly expanded oral spines, the bases of which should communicate with the fertile zoëcium on each side of the operculum. I cannot claim to have proved this to

be the case, though I have obtained some evidence pointing in that direction.

Dr. G. M. R. Levinsen has kindly given me permission to allude to his very interesting observation (which he proposes to publish hereafter), that in *Alysidium parasiticum*, Busk,¹ the ovicells develop as two arched, hollow valves, corresponding with the oral spines which occur on the ordinary zoëcia. I do not at present know what conclusions Dr. Levinsen deduces from this observation, with which I was acquainted before making my own on *E. episcopalis*.

In *Heteroœcium amplexans*, Hincks² has described a *Membranipora*-like Cheilostome, in which the ovicell is constituted by a number of spines placed distal to the operculum, which meet in a *Cribrilina*-like manner, and form a structure which, in other respects, resembles a normal ovicell.

Calvet (1900, pp. 57, 58, 132, pl. ii, fig. 14) states that in *Bugula* there is a communication-pore between the ovicell and the distal zoëcium.

It follows from the account given by Calvet and others, that the cavity of the ovicell, internal to its inner layer, is an external space which is overarched by the double wall of the ovicell. This is obviously true in a case like that of *Mucronella coccinea*, where the most distal oral spines of the fertile zoëcium are actually inside the cavity of the ovicell.

Further investigation is necessary to decide the morphological nature of the Cheilostome ovicell. The existence of three possibilities is generally recognised:—(1) That the ovicell belongs to the fertile (proximal) zoëcium; (2) that it belongs to the distal zoëcium; (3) that it is a modified individual, as believed by Nitsche and others. The second possibility would seem to be indicated by Calvet's observation above referred to. The relation of the operculum of the fertile zoëcium to the ovicell, the occurrence of the "internal

¹ 1852, p. 14.

² 'Ann. Mag. Nat. Hist.' (5), viii, 1881, p. 129, pl. iii, fig. 7; *ibid.* (6), ix, p. 332.

ovicells" found in certain species of *Flustra* and elsewhere, and in particular Dr. Levensen's account of *Alysidium parasiticum*, seem to be in favour of the view that the ovicell is a part of the fertile zoëcium.

In his original account of *Flustra militaris*,¹ Waters suggests that this species is allied to *E. episcopalis*, calling attention to the resemblance between the ovicells of the two forms, but noting the existence of differences between the opercula. The two species agree in the great length of their zoëcia and in their rosette-plates; but a striking difference is seen in the frontal surface, which is membranous in *F. militaris*, except for a small proximal calcified region. Parietal muscles appear to be inserted into this membrane, as in an ordinary *Flustra*. The two strong suboral spines of *F. militaris* may correspond with the two projections which in *E. episcopalis* cut off the median pore. If the frontal surface has really the importance in classification attached to it in the present paper, I see no way of admitting the affinity of the two species (which undoubtedly resemble one another), except by assuming either that *F. militaris* is a species which has secondarily opened its compensation-sac, or that *E. episcopalis* is a modified *Flustrine* form. The material at my disposal is not in a condition which allows me to make a further examination of these points.

IV. THE MORPHOLOGY OF THE COMPENSATION-SAC AND OF THE OPERCULUM.

I have come to the conclusion that the evolution of the compensation-sac has not been identical in all Cheilostomes which possess that structure, but that in some cases it has been formed by the overarching towards the middle line of a series of marginal spines in such a way as to cover the primitive frontal membrane.² The evidence for the occurrence of this process is as follows:

- (1) Many species of *Membranipora* exist in which the

¹ 'Ann. Mag. Nat. Hist.' (5), xx, p. 93.

² See p. 282.

arrangement of the marginal spines foreshadows the condition above indicated.

(2) The Cribrilinidæ are transitional from *Membranipora* to some at least of the Lepralioid genera.

(3) The arrangement of the muscles connected with the compensation-sac is derivable from the condition found in *Membranipora*.

(4) The study of the primary zoëcium.

(5) Palæontological evidence.

The view that the Cribrilinidæ are intermediate between the Flustrina and the Escharina is not a new one. Smitt (1868, i, p. 401) states explicitly that the frontal bars of *Membraniporella nitida* are homologous with the free marginal spines of *Membranipora lineata*; and in his next paper (1868, ii, p. 48) he shows that *Cribrilina* marks a further transition to the Escharines. He leaves *Membraniporella* in the Flustrina, while placing *Cribrilina* in the Escharina. Hincks, on the contrary (1880), places the two genera in the family Cribrilinidæ.

But although taking this view of the intermediate position of the Cribrilinidæ, Smitt was not in a position to show in detail how the Flustrine zoëcium could be modified into an Escharine zoëcium. The compensation-sac enters into the question as supplying the clue necessary for the solution of the problem.

The foregoing instances have given some idea of the relations of the compensation-sac in its fully developed form. I next proceed to the proposition that the compensation-sac of some Lepralioid genera has been derived from a *Membranipora*-like condition through a stage similar to that of existing species of *Cribrilina*.

(A) Flustrina.

Flustra pisciformis, Busk.¹—Fig. 4 shows the general anatomy of a young zoëcium of this species. In the lateral

¹ 1852, p. 50.

walls the calcareous matter is in two layers separated by a chitinous lamella,¹ as described by Nitsche (1871, pp. 421, 455). Each zoecium thus has its own calcareous lateral walls, distinct from those of its neighbours. In two regions, respectively proximal and distal to the broadest part of the zoecium, the lateral wall is thickened at its frontal edge. At the proximal narrow end the calcareous wall becomes deficient, and the terminal partition wall, which is thicker than any of the others, belongs to the proximal zoecium of the two which it divides. This accords with Levinsen's statement (1891, p. 251) that in many Cheilostomes the terminal wall is single, while the lateral walls are double, so that the longitudinal rows of zoecia can be isolated from one another by boiling with caustic potash. The frontal surface is entirely membranous; the operculum is merely a part of this membrane, and has no basal sclerite. When the operculum is open (fig. 3) it is seen to have a vertical flange, produced into lateral points, as in *Euthyris*. A similar arrangement is described by Nitsche² in *Membranipora membranacea*. The ocluser muscles (*occl.*) are inserted into these points, and originate, as in *Euthyroides episcopalis*, from the basal wall. A pair of strong parieto-vaginal muscles (*p. v. m.*) pass from the tentacle sheath to the basal wall, and a smaller pair (*p. v. m.'*) connect the tentacle sheath with the frontal wall; but none pass to the vertical walls (cf. Calvet, 1900, p. 199). A pair of strong parieto-diaphragmatic muscles (*p. d.*) spring from the basal wall, just internal to the origin of the ocluser muscles, and are inserted into the diaphragm or vestibule. The retractor muscles (*r. m.*) of the polypide have their origin in one of the corners of the fish-tail-like proximal end of the zoecium. About four

¹ In a recent paper by Schulz ('Arch. f. Naturg.,' lxvii, Bd. i, Heft 2, 1901, p. 118, pl. vi, fig. 4) this chitinous lamella is figured as a much thicker layer than anything I have ever seen. The species investigated by Schulz was *M. "membranacea"* (= *M. monostachys*, Busk; cf. Levinsen [1891], p. 277).

² 1871, p. 422.

groups of parietal muscles (*p. m.*) are seen, of which the distal group (*p. m.*¹) originates from the basal wall, and the rest from the vertical walls, close to their junction with the basal wall.

These arrangements are moderately constant in all the zoöcia. The distal group of parietal muscles is always strong, and is commonly stronger than the others. The number of groups does not vary much, but it is important to notice that the distal group may (rarely) originate from the base of a vertical wall, and conversely that the other parietal muscles may originate from the basal wall. The distal parietal muscles thus clearly belong to the same series as the rest.

The mode of action of the parietal muscles has been well described by Nitsche (1871, p. 426), who showed that the pressure on the fluid of the body-cavity due to their contraction was the main cause of the protrusion of the polypide. A more detailed account of the same process is given by Calvet (1900, p. 63).

F. pisciformis has no distinct divaricator muscles. The opening of the operculum is probably largely due to the fluid-pressure brought about by the contraction of the parietal muscles generally; but it seems to me highly probable that the distal group of these muscles, by pulling on the membrane with which the base of the operculum is immediately continuous, may have a special effect in opening the operculum. I regard the distal parietal muscles as the starting point from which the divaricator muscles of *Euthyris* and its allies have been derived.

Flustra papyrea, Pall.—This species is very similar to *F. pisciformis* in essential respects. The more noteworthy differences are (1) the parietal muscles are more numerous, usually numbering about six to seven groups on each side;¹ (2) while each of the ordinary groups consists of not more than two or three fibres, the distal group is a much broader and more definite muscular band, composed of a considerably larger number of fibres; (3) the distal as well as the other

¹ They are shown in my paper (1892), pl. ii.

parietal muscles originate typically from the basal end of the lateral walls. The operculum resembles that of *F. pisciformis*.

I have observed the occurrence of typical parietal muscles in five other species of *Flustra*.

Membranipora.—The operculum and the parietal muscles in *M. membranacea* and other forms which have been examined are essentially *Flustrine* in their arrangement. They have been described by Nitsche (1871, p. 426) and others.

Farciminaria hexagona, Busk.¹—The whole of the very long frontal surface is membranous. The operculum is like that of *Flustra pisciformis*, with no basal sclerite, and with a marginal vertical flange prolonged into two lateral points, into which the ocluser muscles are inserted. The orifice is markedly bilabiate, the operculum with the vestibule opening precisely like a clasp-purse, of which the marginal flange of the operculum forms the supporting rim of one half, while the distal edge of the zoecium supports the other half. There are about six groups of parietal muscles on each side, the distal group probably acting as in *Flustra pisciformis*.

The next few species are ordinarily placed with the *Cellularina*, but their affinities appear to me to be with the *Flustrina*.

Dimetopia spicata, Busk² (fig. 5).—This is an instance of a dendritic form, in which the lateral zoecial walls constitute the greater part of the exposed surface of the funnel-shaped zoecium. The frontal membrane is small, and is situated at the broad end of the funnel, the edge of which is surrounded by seven or eight hollow calcareous spines. These originate from a massive calcareous ring which is notched externally at the base of each spine to permit the cellular tissue of the spine to communicate with that of the general body-cavity.

¹ Busk (1884), p. 51.

² Busk (1852), p. 35; MacGillivray, 'Prodr. Zool. Vict.,' Dec. v, 1880, p. 33.

The lumen of the spine has a characteristic globular dilatation at the point where it crosses the calcareous ring. The calcareous wall of this dilatation is completed externally by the outer wall of the zoëcium. The bases of the spines are connected with one another by a thin calcareous web, while part of the frontal membrane may project just beyond the web, internal to the bases of the spines.

The zoëcia are arranged back to back in pairs, new zoëcia (z.) originating from the axial side of their distal end. The orifice is close to this region, on the axial side of the frontal wall (fig. 5). The ocluser muscles (*occl.*) originate from the lateral walls. In correlation with the reduction of the length of the frontal membrane, there is but a single pair of parietal muscles (*p. m.*).

Bicellaria grandis, Busk, var. *producta*, MacGillivray¹ (fig. 1).—This is another example of an infundibuliform zoëcium. The frontal membrane is obliquely placed, and is of restricted extent. The orifice is at the extreme outer end of the membrane, and the zoëcium is prolonged beyond it into a lobe which bears three or four very long spines. On the axial side of the zoëcium is a calcareous thickening² which surrounds the communication-pores (*c. p.*). The zoëcia have the alternate arrangement characteristic of the genus, and in most cases the calcareous thickening includes two sharply marked rings, one for the alternate zoëcium of the other side of the branch, and the other for the next zoëcium on the distal side. In fig. 1, however, there are three calcareous rings, the zoëcium being the one which precedes a bifurcation, and having consequently to give rise to two distal zoëcia. A peculiarity of this species, which I have not found in other species of *Bicellaria*, is that the proximal part of the "aperture" becomes filled in with a thin calcareous film (*crypt.*) which ends in a hook-like point. I am unable to state with certainty what is the function of this hook, but I think that its distal edge forms

¹ 'Prodr. Zool. Viet.,' Dec. vi, 1881, p. 38.

² Cf. Goldstein, 'Trans. Proc. Roy. Soc. Viet.,' xviii, p. 43.

a guide over which the tentacle sheath works in the movements of protrusion and retraction. I have not definitely settled whether the calcareous film is a superficial calcification or an internal cryptocyst, but the existence of a delicate, free, vertical edge all round the "aperture" probably indicates that there is living tissue adjacent to it (which I believe that I have been able to make out), and that the film is of the nature of a cryptocyst.

The operculum is like that of *Flustra*, and there is a single pair of parietal muscles, as in *Dimetopia*.

Bugula neritina,¹ L. The aperture is elongated, and occupies the greater part of the length of the zoecium. The parietal muscles are arranged in a series of *Flustra*-like groups, which lie very close to the lateral walls of the zoecium.

(B) *Cribrilinidæ*.

It has long been known that the characteristic frontal wall of certain *Cribrilinidæ* develops as a series of marginal spines, originating from the periphery of the aperture and converging towards one another until they meet, and so form a calcareous roof in which the intervals between the bars remain either as simple slits or as a series of pores. An excellent account of this process is given, for instance, by Hincks (1880, p. 199).

It is easy to see, by an examination of dry specimens of various *Cribrilinidæ* (e. g. *C. annulata*) that the operculum is not continuous with the calcareous frontal wall, but with the underlying frontal membrane. This may be observed either in immature zoecia or in those from which the wall formed by the union of the bars has been broken away. Goldstein (1880, p. 48) has made the interesting observation that in *C. monoceros* (?) a Rotifer swam into the space between the bars and the frontal membrane. Although this is not expressly stated, it presumably entered at the distal

¹ Busk (1852), p. 44.

end of the series, between the bars and the base of the operculum.

The growth of these bars can easily be studied in the common *Membraniporella nitida*,¹ Johnst. The young zoëcium is completely *Membranipora*-like, even in the character of its operculum. The bars are hollow calcareous spines, which originate as a series of crenulations from the edge of the vertical wall of the zoëcium, each of the future bars being indicated by a round bay of the edge. By the closure of this bay the commencement of a tube is formed, which arches over the frontal membrane in such a way as to leave a considerable space between itself and the membrane. The slits between the bars are probably permanently open. Another point which is worth notice is that the calcareous bars are part of a series of which the distal ones persist as the oral spines. In *Cribrilina annulata* the bars originate in the same way, but the intervals between successive bars persist as a series of pores which probably remain open.

Cribrilina philomela, Busk.²

Dry specimens show at once that the operculum is continuous with the original frontal membrane,³ which becomes covered over in the same way as that above indicated (fig. 44). The fully developed zoëcium (fig. 8) is covered in front by a series of alternate hollow bars, the lumen of each of which is for the most part narrow, but dilates into a rounded end near the middle line of the zoëcium. The rounded end lies more superficially than the rest of the cavity. The outer wall of each bar is considerably thicker than the inner wall.

The operculum is *Flustra*-like, with no basal sclerite, but

¹ Hincks (1880), p. 199.

² 1884, p. 132.

³ This is shown in a diagrammatic longitudinal section of a *Cribrilina* figured by Canu (1900, p. 441, fig. 53, iii), where the roof formed by the united calcareous bars or "costules," is seen to be entirely independent of the operculum.

it is of firmer texture than in the preceding forms. It has the usual lateral projections of the vertical flange for the insertion of the occlusor muscles. Condyles are barely indicated, but they can just be made out as two slight knobs distal to the proximal broadest part of the operculum.

A Flustrine series of groups of parietal muscles¹ occurs (fig. 9), among which a stronger group (*p. m.*) on each side, immediately proximal to the operculum, probably acts as a divaricator, as in *Flustra*.

A few of the zoëcia are modified as vicarious avicularia. The zoëcium is normal, but the operculum is longer than usual, and the occlusor muscles originate from as much as the distal two thirds of the basal wall. These zoëcia are provided with typical parietal muscles. Polypides are absent in most parts of the preparation, but in several cases the vicarious avicularium possesses a polypide. These modified zoëcia are not provided with ovicells, which occur, here and there, on the ordinary zoëcia.

Cribrilina radiata, Moll (fig. 7).

This species, which differs in important respects from *C. philomela*, is considered in the following section (p. 326).

(c) Lepralioid Genera.

Under this heading I shall consider not only some of the genera which are usually referred to the *Escharina*, but a certain number of the branching forms at present included in the *Cellularina*.

Umbonula verrucosa,² Esper (fig. 11; see also the diagram, fig. 12).

The frontal membrane of this handsome species is permanently Flustrine; but it becomes overarched by a strong

¹ The parietal muscles of *Membraniporella nitida* are figured by Smitt (1865), pl. vi, fig. 1; those of *Cribrilina figularis* by Jullien (1888), 2, pl. x.

² Hincks (1880), p. 317.

calcareous roof, which extends to about the middle of the orifice, where it ends in a prominent shoulder on each side, rising in the middle line into a massive suboral umbo. This bears a strong avicularium at its base, immediately over the entrance to the compensation-sac. The rounded mandible, when closed, is directed towards the end of the umbo, and then lies in a vertical plane, transverse to the long axis of the zoëcium. The umbo, which probably belongs to the avicularium, is supported by a series of radiating buttresses of the frontal shield, and between them are deep pits, the marginal areolæ (*ar.*), closed by a layer of living tissue or epitheca.

Fig. 11 shows the commencement of the calcification of the frontal shield. The lateral partition-walls consist of a thin chitinous lamella, with a layer of calcareous matter on each side, belonging respectively to the two zoëcia separated by the wall. At a level a little lower than the free edge, the lateral and proximal partition-walls give off a calcareous film, which in the young specimen figured reaches no further than the edge of the frontal membrane. This film is pierced by a series of round holes (*p.*), one of which corresponds to each interval or areola (*ar.*) between two of the future buttresses. These holes establish a continuity between the living tissue of the body-cavity and that which occurs on the outside of the frontal shield. Beyond the origin of the frontal shield the vertical wall splits into two membranous lamellæ, one of which passes over each of the contiguous zoëcia to form its epitheca, and becomes continuous with the free edge of the frontal shield (fig. 12). The frontal membrane (*f. m.*) becomes covered by a continuation of this process—in other words, by the formation of a crescentic fold, of which the deeper lamella is calcareous, and the superficial layer is composed of a living membrane. In the incompletely calcified zoëcium the edge of the crescentic fold is always membranous. From the deep lamella rise the radiating ridges which form the buttresses.

The polypide is still young in fig. 11. With the distal end

of its tentacle sheath are connected the two "opercular glands,"¹ while somewhat more proximally are inserted the conspicuous parieto-vaginal bands (*p. v. m.*). The operculum is of delicate Flustrine structure, and has no special divaricators. Of the strong occlusor muscles (*occl.*), that of the (apparent) left side is seen to originate from the distal vertical wall, and that of the right side from the lateral wall. A series of groups of parietal muscles (*p. m.*) originate from the lateral and proximal walls, and are inserted into the frontal membrane.

There can, I think, be little doubt that the space below the calcareous roof is the homologue of the similar space in *Cribrilina*. The main difference between the two spaces is that that of *Cribrilina* is covered by a series of originally separate bars, while in *Umbonula* it is from the first a continuous crescentic film. The marginal pores,² which are the only ones that occur in the film, have, however, precisely the same relations as the communications between the cavities of the hollow *Cribrilina* spines and the general body-cavity. I regard the covering of the compensation-sac in *Umbonula* (fig. 50) as having been derived from a *Cribrilina*-like condition (fig. 44) by the lateral fusion of the spines with one another, the edges of the spines being indicated by the buttresses, and their cavities by the marginal areolæ and by the pores leading from those spaces to the general body-cavity. This involves the necessity of assuming that the outer calcareous layer of the *Cribrilina* spines is now represented by an uncalcified membrane, the epitheca. The very common occurrence of marginal areolæ in *Escharine* forms may be appreciated by turning over the plates of almost any paper dealing with a collection of recent or fossil forms.

¹ Cf. Waters (1892), p. 272 ("gland-like bodies"), and Calvet (1900), p. 200.

² The relations of these pores are well shown in a figure given by Neviani ('Boll. Soc. Geol. Ital.,' xv, 1896, p. 24).

Umbonula pavonella, Alder (= *Mucronella*
pavonella, auctt.¹).

The structure of the adult zoëcium (fig. 10) closely resembles that of *U. verrucosa*, and I venture to place the two species in the same genus. There is no umbo nor median avicularium, but the frontal shield ends in a rounded median lobe which is not raised above the level of the rest of the shield. Each of its lateral shoulders (*f. sh. d.*) bears a conspicuous avicularium (*avic.*). Its free surface rises into a series of radial buttresses, and the areolæ (*ar.*) between these communicate with the general body-cavity by pores arranged as in *U. verrucosa*. My preparations show with great distinctness that cords of living tissue (*v*) traverse these pores, and unite to form a continuous sheet of living substance (indicated by the brilliantly stained nuclei) which overspreads the entire frontal shield even in old zoëcia. The basal wall is not provided externally with a living membrane.

It is well known that the front surface of Cheilostome zoëcia may alter greatly with age. The original pattern may become lost, the thickness of the wall may increase very greatly, and the orifices of the zoëcia may finally be completely covered by secondary calcification. All these changes can be understood when it is realised that the frontal shield of an Escharine Cheilostome is covered by living tissue. Milne-Edwards (1836, p. 27), in discussing the alterations which take place with age in Escharine forms, came to the conclusion that the calcareous matter was a living tissue which grows like a bone. Goldstein (1880, p. 48) has stated that in *Mucronella ellerii* the brilliant scarlet colour "seems to be located in a fleshy epidermis, with which the stony polyzoary is coated." This is the "epithea" of many authors, commonly seen in dry preparations as a membrane overspreading the calcareous frontal shield, and consisting, in reality, of an external cuticle and subjacent living tissue.

¹ Hincks (1880), p. 376.

The existence of the epitheca is responsible not only for the ordinary form of secondary calcification, but it explains the condition so commonly found in *Cellepora*, where new zoëcia originate on the free surfaces of the old ones. This appears to be the result of the separation of the epitheca from the calcareous wall, the subjacent space (which is morphologically part of the body-cavity) increasing in size and forming the body-cavity of a new zoëcium.

Lepralia pallasiana,¹ Moll (Pl. 17, fig. 41).

The frontal wall of the young zoëcium is at first Membranipora-like. Calcification begins at the proximal end by the formation of a thin calcareous film, which is perforated by large, uniformly spaced pores. This film is formed *in situ* beneath a delicate epithecal membrane. The process proceeds with considerable rapidity until the whole of the frontal wall is calcified, with the exception of the operculum, between which and the distal end of the zoëcium occurs a portion of the frontal wall, with a single row of large pores. In the earlier part of this process the polypide bud is small, and is at the proximal end of the zoëcium, being connected with the membranous frontal wall by a cord of cells. This becomes hollowed out to form the tentacle sheath (fig. 41), at the distal end of which appear two thickenings, the opercular glands (*op. gl.*). When the calcification is completed, the compensation-sac (fig. 41, *c. s.*) begins to be obvious as a well-marked cavity, extending from the base of the operculum beneath the calcareous frontal wall, and at first much shorter than the operculum. Into the floor of the sac are inserted the parietal muscles (*p. m.*), which, in the immature zoëcium, radiate even from distant parts of the lateral walls of the zoëcium.

The sac rapidly grows to such an extent that it underlies the whole of the frontal wall, the parietal muscles thereby acquiring an arrangement similar to those of *Cribrilina*.

¹ Hincks (1880), p. 297.

The process is identical with that which has been described above in *Euthyroides episcopalis*. The fact that the wall of the compensation-sac becomes tightly pressed against the lateral wall of the zoëcium makes it difficult to see its outline in the old zoëcia; and the same applies to many other Cheilostomata. But in a decalcified preparation the characteristic parietal muscles can be seen, even in old zoëcia, with the utmost distinctness.

The operculum articulates with two small condyles which are situated just on the distal side of its proximal broader part. The appearance of two linear longitudinal submarginal sclerites (fig. 41) is due to the special development of the vertical flange into two lateral ridges, the distal connecting part of the flange being less developed. The occlusor muscles (*occl.*) are inserted into these lateral ridges, and they originate low down from the vertical walls of the zoëcium, either from the lateral walls or from the distal wall. The divaricator muscle is the distal group of parietal muscles on each side. I am not certain whether the insertion of these is directly into the base of the operculum or into the floor of the compensation-sac close to the operculum.

The small, non-porous, suboral region of this species may be taken to indicate the former presence of a suboral avicularium¹ (as in *Umbonula verrucosa*). This view is confirmed by the fact that a few of the zoëcia actually possess a small avicularium with a semicircular mandible, which, when closed, lies horizontally, and points away from the operculum.

L. pallasiana is one of the forms which has been specially studied by Calvet (1900), many of whose statements I can confirm, although he has not observed the compensation-sac. In the younger zoëcium shown in his pl. xiii, fig. 20 (*L. foliaceae*), it appears to me that he indicates the development of the compensation-sac as an ectodermic invagination at the proximal end of the operculum. In his pl. vi, fig. 1,

¹ Waters (1883, p. 439) calls attention to the common occurrence of the suboral avicularium in Cheilostomes.

which in other respects gives a good idea of the general structure of the zoëcium, I think he has put the insertion of the occlusor muscles too near the proximal end of the operculum.

Lepralia dorsiporosa, Busk¹ (fig. 45).

In one respect this species appears to me to show more primitive characters than *L. pallasiana*. The distal prolongations (*f. sh. d.*) of the frontal shield do not completely surround the orifice, the point where they meet being commonly indicated by a slight emargination on the distal side of the zoëcium. Each distal prolongation typically bears an avicularium (*avic.*).

The compensation-sac is seen with great distinctness in a decalcified preparation. It underlies the whole of the frontal wall on the proximal side of the operculum, and it is provided with typical parietal muscles. The distal group on each side is specially strong, and it appears to me that the part of the compensation-sac immediately adjacent to the base of the operculum, into which these muscles are inserted, is somewhat fascia-like, an arrangement which confirms the view that the muscles function as divaricators. The condyles (*cond.*) are well-developed recurved hooks.

Lepralia sincera, Smitt.²

In this species, for specimens of which I am indebted to Dr. Levinsen, the compensation-sac and its muscles resemble those of the preceding species. The distal shoulders of the frontal shield extend no further than about the middle of the operculum. One of these shoulders is occasionally provided with an avicularium.

Lepralia haddoni, n. sp. (figs. 38, 39).

Zoarium encrusting or bilaminar (Escharine). Zoëcia in regular longitudinal rows, each about 700—900 μ long and about 370 μ broad. Surface covered by a distinct epitheca.

¹ 1884, p. 143.

² 'Öfv. k. Vet.-Ak. Förh.,' xxiv (Bihang, 1867), 1868, pp. 28, 177.

Proximal part of the frontal shield with numerous large pores, which do not extend to the distal end of the zoëcium. The oral end of the zoëcium may be somewhat raised above the general surface of the flat frontal shield. A rounded suboral lobe occurs, and may have almost the appearance of a mucro. Ordinary orifices and opercula somewhat longer than broad, with a concave proximal margin, $210\text{--}250\ \mu$ broad; fertile zoëcia with trifoliate orifice and operculum, $320\text{--}340\ \mu$ broad. External ovicells only represented by the somewhat hood-like calcareous wall at the distal end of the zoëcium. An avicularium, with long linear mandible, commonly occurs on one side of the orifice. Basal surface smooth, its intersection with the four vertical walls forming a regular oblong. Pore-chambers absent.

Torres Straits, A. C. Haddon collection, 1888-9.

This species somewhat resembles *L. feegeensis*, Busk, but differs from it in the absence of external ovicells. It is of interest in throwing light on the meaning of the occurrence of dimorphic opercula. The outline of the ordinary operculum (fig. 38) is not unlike that of the A-opercula in *Euthyris oblecta*. The B-opercula are much wider, and are trifoliate. In either kind of zoëcium there may be an avicularium on one side of the orifice, its acute mandible sloping, when closed, towards the distal end of the zoëcium. The compensation-sac is present in both kinds of zoëcia, and has typical parietal muscles. In some zoëcia it contains long wavy filaments, which may be parasitic Algæ or Fungi. In the zoëcia with trifoliate operculum an ovary (fig. 39) is developed at the basal end of one of the lateral walls. A spherical ovisac, with delicate walls, makes its appearance at the distal end of the zoëcium, on the basal side of the operculum and tentacle sheath. Into this sac are inserted muscle-fibres, which radiate to it from the adjacent parts of the basal and lateral walls. In one zoëcium an egg has matured and has become filled with yolk, its diameter nearly equalling that of the zoëcium. This egg has passed into the

interior of the spherical ovisac, while the ovary still remains in its original position.

My preparations do not enable me to trace the origin of the egg-containing sac, which may, however, be a diverticulum of the vestibule, as described by Calvet (1900, fig. 42, on p. 266) in *Lepralia pallasiana*.

The conclusion pointed to by these facts is that the dimorphism of the opercula indicates the derivation of the species from forms provided with ovicells; in which, as is well known, the operculum of the fertile zoëcium is commonly larger than that of the other zoëcia.

Flustra cribriformis, Busk.¹

This species is introduced at this point in further illustration of the morphology of the ovicell. Its frontal wall is, of course, typically Flustrine. The ovicell is of the type usually described as "internal,"² and is merely represented by a more or less hemispherical bulging of the distal zoëcial wall into the next zoëcium. Into this space projects a spherical vesicle, which lies just beneath the vestibule, as in the foregoing species. The polypides are retracted to the extreme proximal end of the zoëcium. In some cases I have been able to detect a very small ovary in one of the proximal corners of the zoëcium, near the basal wall; but in consequence of the position of the polypides I cannot say whether the ovary is parietal or attached to the polypide. The egg enters the spherical sac, in some manner which has not been observed, while it is still very small. It receives its yolk while in the sac, and when it is full grown it is so large that with its investing sac it occupies as much as half of the body-cavity.

Schizoporella linearis,³ Hass. (figs. 48, 52).

The characteristic "sinus" or projecting proximal tongue of the operculum of this genus is very similar to the part of the operculum which in certain species of *Lepralia* occurs

¹ 1852, p. 51; 1884, p. 58.

² Cf. Levisen (1891), p. 275.

³ Hincks (1880), p. 247.

on the proximal side of the condyles. It appears to me that the function of the tongue is partly to close the aperture of the compensation-sac, and partly to give suitable leverage for the action of the divaricator muscles.

S. linearis is characterised by the possession of a pair of suboral avicularia, with pointed mandibles. Hincks (1880, p. 251) has described in this species a form of "oœcium," associated with a rudimentary zoœcium. My preparations show clearly that these supposed ovicells (fig. 48, *avic.*) are in reality gigantic suboral avicularia,¹ of the type well known in *Retepora monilifera*, var. *munita*, Hincks.² The greatly swollen region distal to the mandible is occupied by the enormous occlusor muscles, which differ only in size from those of the ordinary avicularia, their relation to which is further shown by the fact that a single gigantic avicularium may lie obliquely across the zoœcium instead of longitudinally as in fig. 48. The compensation-sac (fig. 52, *c.s.*) of the zoœcia is small, and even in zoœcia with fully mature polypides is commonly no larger than in the specimen figured.

The sinus of the operculum (fig. 52) fits into a corresponding emargination in the frontal shield. At a deeper level are seen the two condyles (*cond.*) which constitute the hinge. The frontal wall is of considerable thickness, and its distal margin, in the neighbourhood of the sinus, is bevelled off in precisely the way noticed in *Euthyris clathrata* (fig. 26). This results in the formation of a groove, the outer end of which is closed by the sinus of the operculum, while the inner end (fig. 52, *x*) is distinctly larger. When the operculum is open, its plane must be approximately parallel to the part of the bevelled surface which lies in the middle line. A short tube is thereby formed by which water can enter or leave the compensation-sac.

The compensation-sac develops as in *Lepralia pallasiana*. The calcification takes place beneath the epitheca,

¹ Waters (1885, p. 6, and 1892, p. 274) has previously stated that this is the case.

² 'Ann. Mag. Nat. Hist.' (5), i, 1878, p. 361.

since a layer of nuclei can be seen superficial to it. The parietal muscles are very delicate. The occlusor muscles originate from the distal wall, but I have not certainly seen divaricator muscles.

Schizoporella sanguinea, Norman (fig. 46).

This is the form described by Waters,¹ in his paper on the Polyzoa of Naples, as *Lepralia pertusa*, var. *sinuata*.

The operculum is rather stronger than in *S. linearis*, and it has a well-marked marginal sclerite which is distinctly thickened at the points where it articulates with the condyles. The compensation-sac (fig. 46, *c. s.*) is beautifully shown in specimens from which the basal wall has been removed. The parietal muscles radiate into its lateral walls, and the external parts of its basal wall, from the lateral and proximal walls of the zoecium. The distal parietal muscles (*p. m.*'), which stretch transversely across the zoecium, probably act as divaricators.

Dry specimens show the epitheca, stretched over the tubercles of the reticulately thickened frontal shield.

Schizoporella australis, Haswell.²

This species is characterised by having an elongated avicularium on the proximal side of the orifice, with an acute mandible directed obliquely towards the proximal end of the zoecium when closed. The condyles are strong, and have denticulated edges (fig. 47). The continuity of the floor of the compensation-sac with the sinus of the operculum is clearly seen in the preparations. The sac resembles that of *S. linearis*.

Urceolipora nana,³ MacGill., 1881.

The operculum is *Schizoporella*-like. At its proximal border opens a large compensation-sac which extends nearly

¹ 'Ann. Mag. Nat. Hist.' (5), iii, 1879, p. 31, pl. viii, fig. 5.

² 'Proc. Linn. Soc. N. S. Wales,' v, 1881, p. 41.

³ MacGillivray, 'Prodr. Zool. Vict.,' Dec. xi, 1885, p. 19; Busk (1884), p. 82 (as *Calymnophora lucida*).

to the proximal end of the zoëcium, a great part of the cavity of which it occupies. The two calcareous spines at the sides of the orifice have the effect of keeping the conspicuous epitheca, which invests all parts of the zoarium, stretched out at some distance from the wall of the flask-shaped zoëcium. The curved lines shown by Busk (1884, pl. xxxii, fig. 3 *b*) on the sides of the zoëcia indicate the edges of the thicker lateral calcified walls, and are probably the actual junction of the lateral and frontal walls. Their level corresponds nearly with that of the floor of the compensation-sac, which has parietal muscles. The sac originates with great distinctness as an invagination from the proximal border of the operculum.

Smittia trispinosa, Johnst., var. *arborea*, Lev.¹ (fig. 42).

The specimens here referred to were sent to me, determined as above, by the Upsala Museum. Fig. 42 shows that there is a large compensation-sac (*c. s.*), the opening of which is overhung by a median lobe of the frontal shield. The condyles (*cond.*) are at a much deeper level, and are long, recurved denticles, which are crossed by the submarginal lateral sclerites of the operculum. They clearly form the hinge on which that structure moves. Typical parietal muscles are present. This form can hardly be regarded as a very typical *Smittia*.

Smittia reticulata,² J. MacGillivray (fig. 40).

My evidence with regard to this species is not complete. In specimens from which the basal wall has been removed I have just been able to detect a large compensation-sac with walls of great tenuity, into which parietal muscles are inserted. The median denticle (*m. t.*) or "lyrula"³ appears to belong to the suboral avicularium (as in *Rhynchozoon*).

¹ "Bryozoer fra Kara-Havet," 'Dijmphna-Togtets Zool. bot. Udbytte,' 1886, p. 16.

² Hincks (1880), p. 346.

³ Cf. Waters, 'Ann. Mag. Nat. Hist.' (6), iv, p. 14.

The principal interest of this species, in the present connexion, is as an illustration of the group of Cheilostomes in which there is but a single series of conspicuous marginal pores or areolæ (*ar.*).

Catenaria lafontii,¹ Aud. (fig. 49).

I consider this species in this place on account of its possession of a typical compensation-sac; but I shall discuss its affinities later. The zoœcia are very long, curved, narrow proximally, and dilating distally. From the back of the distal end usually originate a pair of new zoœcia (*z.*'), each starting in a calcareous base containing a body-cavity, into which is fitted a chitinous joint (*j.*), which forms the connexion with the younger zoœcium. The oral extremity of the zoœcium is surrounded by a circle of short, hollow spines (*sp.*). The ovicell (*ov.*) is placed obliquely on the distal side of the orifice, and an extremely strong suboral avicularium (*avic.*) guards the entrance to the compensation-sac. There is some evidence that the base of the avicularium has been derived from a single pair of suboral frontal spines. The porous region of the zoœcial wall in the main corresponds with the limits of the compensation-sac (*c. s.*), but a few pores occur outside that region, even on the basal surface.

The compensation-sac is extremely distinct, and is indicated in a figure by Calvet (1900, pl. viii, fig. 9), who does not, however, make any reference to the structure. It is provided with several groups of parietal muscles (*p. m.*). The operculum is placed obliquely, and its base is distinctly continuous with the floor of the compensation-sac, the orifice of which is rather large. Each occlusor muscle (*occl.*) originates from the partition wall between the body-cavity of the zoœcium and the base of the daughter-zoœcium of its own side. I have not distinguished any divaricator muscles.

¹ Busk (1852), p. 14.

Vittaticella cornuta,¹ Busk (fig. 56).

Maplestone² has recently suggested the generic name *Vittaticella* for the "vittate" species of *Catenicella*, the subdivision of which appears to me desirable.

Fig. 56 shows that *Vittaticella* is provided with a well-developed compensation-sac (*c. s.*). The younger zoöcium further illustrates the fact that the development of this structure is typically Lepralioid. The sac is figured by Jullien (1888, 3, pl. xi), in two species of *Catenicella*, in both the young and the adult condition. A wide crescentic opening into the sac is seen in the younger zoöcium (fig. 56) between the base-line of the operculum and the proximal border of the orifice. Amongst the parietal muscles which radiate from the wall of the sac can already be distinguished a distal group (*p. m.*'), the future divaricator muscles. In the next zoöcium the compensation-sac is so large as to underlie nearly the whole of the frontal surface.

Each vitta (*v.*) is a tubular cavity, running longitudinally along the edge of the zoöcium, and bounded externally by a delicate uncalcified membrane. The cavity communicates by a single series of funnel-shaped tubes with the body-cavity.³ In the young zoöcia the whole of this arrangement is filled with a brilliantly staining cellular material; but the structure is identical in the older zoöcia, in which remains of cells may still be made out.

I refer to the morphology of the vittæ below when dealing with *Catenicella hastata*. Their function is, perhaps, to assist in the deposition of the calcareous wall. The zoöcium in this species is strengthened by a great thickening of its edge, which is more pronounced in the region of the vitta than elsewhere. The vitta extends along the whole length of the lateral thickening, and in the more elongated zoöcia it may have as many as ten pores.

¹ Busk (1852), p. 11.

² 'Proc. Roy. Soc. Vict.' (N. S.), xiii, 1901, p. 201.

³ Cf. Waters (1881), p. 318.

The characteristic horns of this species are probably to be regarded as modified avicularian zoëcia. In certain zoëcia one of the horns is replaced by a large normal avicularium.

Catenicella alata, Wyv. Thomson¹ (fig. 53).

The zoëcial structure of *Catenicella* has been described by Busk (1852, p. 4), and by Waters (1883, p. 428). The zoëcium develops on each side three structures, which together form the great wings so characteristic of this species. Taking a case which is not complicated by the presence of the twin zoëcium (cf. fig. 56) which occurs just before a bifurcation of the branch,² the zoëcium is found to be somewhat spindle-shaped, each end passing into a tubular sheath which surrounds the chitinous joint by which it is connected with its neighbours. The basal surface of the zoëcium is extremely gibbous, and projects in this direction far beyond the wings. The frontal surface is nearly flat. The large orifice has, on its proximal side, a scutiform calcareous region bearing five fenestræ closed by membrane. The zoëcium is considerably wider than this scutiform plate, its convex lateral surfaces being overlapped by parts of the wings.

Each wing consists of three parts: (1) a large proximal cavity (infra-avicularian + pedal compartments of Waters), which is almost two thirds of the length of the zoëcium, and is provided on its frontal surface with two large membranous fenestræ, one at each end (fig. 53, *inf. avic.*); (2) a transverse cylindrical cavity, the free outer end of which has a membranous vacuity (*avic.*); (3) a large distal cavity with a single large fenestra, its distal point being uncalcified (*sup. avic.*). The second of these cavities is morphologically an avicularian zoëcium, as is proved by comparison with other species of *Catenicella*.

The five suboral fenestræ perforate the entire thickness of the calcareous frontal wall. At the level of their internal

¹ 'Nat. Hist. Review,' v, 1858, "Proc. of Societies," p. 137.

² Waters terms the solitary zoëcium a "globulus," and the two associated zoëcia a "biglobulus."

openings a sharply marked curved line (*v*) indicates the edge of a calcareous plate similar to that described below in *C. plagiostoma*. The markings between the fenestræ and the operculum are somewhat variable. In a specimen which partly dried up during its preparation, fine slits containing air were noticed, radiating from the fenestræ to the orifice.

The compensation-sac of this species is described by Jullien (1888, 3). It is well developed, but its complete outline is not easily seen in the mature zoëcium in consequence of the fact that its breadth is greater than that of the exposed scutiform frontal wall.

Catenicella plagiostoma, Busk, var. *setigera*,
MacGillivray¹ (fig. 54).

This species is remarkable for the obliquity of its orifice, and for the enormous development of its avicularia, which may have very different forms, even on opposite sides of the same zoëcium (fig. 54). The infra-avicularian compartment (*inf. avic.*) is almost entirely closed by membrane, the single fenestra so formed extending mainly over the side and basal surface of the zoëcium. The supra-avicularian compartment (*sup. avic.*) similarly has membranous walls, a very large fenestra being common to it and the avicularian zoëcium.

The structure of the frontal wall is more easily made out than in *C. alata*. The outer calcareous layer is reduced to a system of conspicuous bars which unite with a calcareous framework surrounding the operculum. The internal calcareous layer is an obliquely oval plate² (*pl.*), which projects towards the proximal end of the zoëcium. This forms part of the roof of the compensation-sac, from which muscles (*p. m.*) can be seen radiating out to the adjacent parts of the wall of the zoëcium. The distal groups of parietal muscles (*p. m.*) appear to act as divaricators.

In a back view of a zoëcium it is seen that the avicularian zoëcium has a very large oblique fenestra proximal to the

¹ 'Prodr. Zool. Viet.,' Dec. iii, 1879, p. 17.

² This structure is described by Waters (1883, p. 429).

mandible, and another distal to the same structure, and common to it and the supra-avicularian compartment. From various parts of the membrane covering the dorsal fenestræ rise the spine-like processes to which the variety owes its name.

Catenicella hastata, Busk¹ (fig. 55).

The supra-avicularian compartment (*sup. avic.*) is calcified except at its extreme tip, the walls being perforated by small pores. It forms a broad, flattened spine at the upper lateral corner of the zoëcium. The avicularian mandible is minute, but the avicularium possesses, in addition to its ocluser and divaricator muscles, a polypide rudiment (*pol.*), as in various other Cheilostomes. I have noticed a similar structure in other species of *Catenicella*. The infra-avicularian compartment (*inf. avic.*) is completely divided into two. The outer wall of both these cavities is usually completely calcified, but the proximal one may have a slit-like membranous fenestra, which is usually lateral. Between the proximal portion, designated by Waters (1883, p. 428) the "pedal compartment," and the zoëcium, was noticed a row of three communication pores (*c. p.*). A comparison with *Vittaticella* suggests that the vittæ shown in fig. 56 are the last remains of the pedal compartments with their communication pores. This view has already been maintained by Waters (p. 428), and, though not quite in the same manner, by Jullien (1888, 3). I have no suggestion to make with regard to the function of the lateral "compartments" in *Catenicella*.

The frontal wall has from seven to nine small fenestræ, from which tubular cavities pass transversely towards the middle line. The arrangement is strikingly *Cribrilina*-like, and the resemblance is intensified by the existence of irregular slit-like cavities alternating with the fenestræ, which suggest an incomplete lateral fusion of frontal bars.

The compensation-sac is usually clear. Its floor appears to be deeply pigmented, but this may be the result of the

¹ Busk (1852), p. 7; 1884, p. 10.

method employed, this preparation being the only one which I have made with Mayer's cochineal tincture. The species seems, however, to have a special development of pigment, as indicated by Maplestone.¹ The compensation-sac develops as in *Vittaticella*.

Catenicella lorica,² Busk.

The remarkable fertile zoëcia which characterise the genus *Catenicella* are well seen in this species to be provided with a large compensation-sac, with strong parietal muscles. The three fenestræ of the ordinary zoëcia appear to perforate only a single thin calcareous layer, the inner layer being completely absent.

Catenicella wilsoni,³ MacGill.

The great size of the fenestra of the infra-avicularian compartment makes it an easy matter to see the outline of the large compensation-sac of this species, and the arrangement of its muscles.

(D) Microporelloid Genera.

Under this heading I consider a few of the forms with a "median pore," though I am by no means certain that all such forms are related to one another.

Calwellia gracilis, Maplestone⁴ (figs. 61, 62).

The zoëcia are in pairs, back to back, the plane uniting the middle line of two zoëcia being at right angles to the

¹ 'Trans. Proc. Roy. Soc. Vict.,' xviii, 1882, p. 49.

² Busk (1852), p. 6.

³ MacGillivray, 'Prodr. Zool. Vic.,' Dec. ix, 1884, p. 30.

⁴ The form which I describe in this paper was figured by Maplestone, without description, in a paper entitled "Observations on Living Polyzoa," in 'Trans. Proc. Roy. Soc. Vict.,' xviii, 1882, p. 48, fig. 9 [the plate has no number]. It has not the triangular shape mentioned by Wyville Thomson (1858) in his original account of *C. bicornis*. It is not the species described by MacGillivray as *C. gracilis* in 'Trans. Proc. Roy. Soc. Vict.,' xxii, p. 128; see also the same journal (N. S.), ii, p. 106.

similar plane of the next pair. The narrow proximal part of each zoëcium extends the whole length of the subjacent pair, in such a way that each zoëcium originates from the next lower one which looks in the same direction as itself.

The two lateral horns are not calcified at their tips (fig. 62, *f*). Below the oblique orifice is a triangular region sloping to the median pore (*m.p.*), which occupies the most projecting part of the zoëcium. The middle line of this region is marked by a suture (*s.*), on each side of which is an "oculiform" pore. Besides the communication pore at each end, by which it is connected with its neighbours in the same longitudinal series, each zoëcium has a pair of distal communication pores placed on the basal side, by which its soft tissues are continuous with those of the narrow proximal half of the next distal pair of zoëcia, while its own proximal half is similarly provided with a pair of pores (*c.p.*) which lead to the two zoëcia of the subjacent pair.

The fully developed compensation-sac (fig. 62, *c.s.*) occupies a large proportion of the body-cavity. Fig. 61 represents a compensation-sac which is about half grown, with its parietal muscles. The sac does not extend to the semi-circular operculum, the straight base-line of which appears to constitute the hinge. It develops in a Lepralioid manner, a small, apparently solid mass of cells appearing beneath the newly formed median pore; and to this mass the parietal muscles radiate from various parts of the zoëcium. Somewhat earlier the uncalcified, oblique, terminal wall of the zoëcium extends as a triangular point as far as the median pore, so that the sac is probably derived from the proximal part of the orifice. The suggestion has often been made (cf. Hincks, 1880, p. 237) that the Microporellid median pore has been formed by the closure of the sinus of a Schizoporella-like ancestor. The above-described immature stage of *C. gracilis* appears to point in this direction.

The occlusor muscles (*occl.*) are easily seen, but I have found no divaricators.

Calwellia sinclairii,¹ Busk (fig. 60).

Waters (1888, p. 17) has described the compensation-sac in this species, as well as in *C. bicornis* and *Urceolipora dentata*, and he correctly states that it opens to the exterior by means of the median pore. I agree with Waters that *C. sinclairii* should be placed in the genus *Calwellia*. It differs from *C. gracilis* principally in the fact that the proximal half of the zoëcium is almost as broad as the distal half, and the zoëcia do not therefore appear to be so definitely arranged in pairs.

The operculum is a segment of a circle somewhat greater than a semicircle, and having a straight base-line. From the middle line of this a longitudinal suture (*s.*) passes to the transversely elongated median pore (*m.p.*), and on each side of the suture is a round fenestra. The suture can usually be traced down the entire length of the zoëcium. The position of the lateral horns of *C. gracilis* is indicated by a fenestra (*f.*), in the neighbourhood of which are one or two smaller pores. The median pore is somewhat crescentic, with an anterior concavity. The lateral edge of the large compensation-sac does not pass to the base of the operculum, but turns sharply inwards at the level of the median pore, and runs into the broad tongue-like lobe of the wall of the zoëcium which gives the pore its crescentic form.

The operculum has a well-marked triangular lobe of its vertical marginal flange (*fl.*), into the apex of which the oclusor muscle is inserted, as in *Flustra*.

Calwellia dentata,² MacGillivray.

It appears to me that this species should be separated from *U. nana*,³ and placed in *Calwellia*, the generic character of which would then have to be amended so far as relates to the arrangement of the zoëcia. In *C. dentata* these are placed

¹ Busk (1884), p. 103.

² 'Prodr. Zool. Vict.,' Dec. xi, 1885, p. 19.

³ As suggested by Waters (1888, p. 10).

back to back, alternately, all the zoëcia looking towards one or the other edge of the branch. The zoëcial characters agree with those of *C. sinclairii*. There is a large compensation-sac, opening by a median pore, which is transversely elongated, but the concavity of which is on the proximal side. Between this and the operculum are two fenestræ, placed as in *C. sinclairii*. In addition to two lateral horns, similar to those of *C. gracilis*, there are three other oral spines placed more distally.

Ichthyaria oculata, Busk¹ (fig. 59).

There can be no doubt that this species was incorrectly placed by Busk in the Bicellariidæ, a family with which it appears to have no affinity. Its more natural position would probably be in the Calwelliidæ,² with which it agrees in the characters of its zoëcium and of its ovicell. The zoëcia are arranged in a double alternate series, all facing in one direction. The median pore (*m. p.*) is circular, and is situated on the axial side of the middle of the zoëcium. It is connected with the operculum by a longitudinal suture (*s.*), on each side of which is a funnel-shaped fenestra, as in *Calwellia*. In correlation with the asymmetrical position of the median pore the abaxial fenestra is much further from the suture than is the other one. There are one or two distal fenestræ, probably indicating the former development of oral spines.

The compensation-sac (*c. s.*) is arranged as in *Calwellia*. It opens by the median pore, and therefore ends at some distance from the operculum. The parietal muscles (*p. m.*) occur as a series of definite groups, as in *Flustra*. Calcification is deficient along a line extending the whole length of the basal side of the zoëcium. The line is narrow for the greater part of its course, but dilates at each end. The older parts of the colony are supported by a calcareous

¹ Busk (1884), p. 46.

² Waters (1888, p. 10) places it in *Calwellia*, a view which seems not unreasonable.

thickening, which runs along each margin of the branch. The retractor muscles of the polypide originate from the two lateral walls. On each side there is a strong fan-shaped group passing to the base of the tentacles, and another passing to the junction of the pharynx with the œsophagus.

Onchoporella bombycina, Busk¹ (not Ell. and Sol.).

Although I have had only dry material of this species to work with, I can state that there is a well-developed compensation-sac, opening by the crescentic median pore, the tongue-like lobe to which the crescentic shape is due being distal, and seeming to be a triangular membrane which curves into the distal border of the compensation-sac (as in fig. 60). The operculum is of very delicate texture. On either side of the orifice, somewhat proximally, there is a short spine, on the proximal side of which is a pore, which is either a simple funnel-shaped deficiency of the calcareous wall, or it consists of two, or even three, funnel-shaped tubes contained in a single longitudinal groove. A similar arrangement is shown in fig. 60 (*f.*). One or two pores of the same kind may continue the line of this groove down the frontal surface of the zoœcium.

The branches have a calcareous marginal thickening, which may have a zigzag course; it is composed of numerous small pieces, an arrangement which clearly gives a certain amount of flexibility to the frond.

Microporella malusii,² Aud. (fig. 63).

The median pore (*m. p.*) has a distal concavity, due to the projection into it of a tongue-like process which is partly membranous. Between this and the straight base-line of the semicircular operculum is a triangular group of pores, the

¹ Busk (1852), p. 52. The species described by Ellis and Solander ('Nat. Hist. Zooph.,' 1786, p. 14), from the Bahama Islands, appears to be a *Euthyris*.

² Hincks (1880), p. 211.

apex of the group pointing to the median pore. Round the edge of the zoëcium is a single line of pores. This arrangement is constant in my Neapolitan specimens. The communication pores open into "pore-chambers" (*p. c.*), as pointed out by Levinsen (1891, pp. 250, 285).¹

The compensation-sac (*c. s.*) is thin-walled, its outline being somewhat heart-shaped when seen from the basal surface. The median pore opens into it considerably on the proximal side of its distal margin, a triangular bay (*corn.*) of the body-cavity indenting the membrane of the compensation-sac in the immediate neighbourhood of the frontal surface. The parietal muscles (*p. m.*) are strong, and are arranged in distinct groups.

The function of the median pore in *M. malusii* has frequently been discussed, and my results do not agree with those of any of my predecessors. Jullien (1888, 4, pp. 36, 39) gives the name "fenestrula" to the median pore, which he describes as forming the narrow end of a funnel ("cornicula"), the other end of which opens into the tentacle sheath. Jullien figures the cornicula in pl. xv, fig. 2, from which it appears to me clear that the funnel is the part of the body-cavity which I have described above (fig. 63, *corn.*) as indenting the distal outline of the compensation-sac.

Pergens (1889, p. 507) does not believe in the existence of Jullien's compensation-sac, and describes the diaphragm or vestibule as opening into the body-cavity and not into the tentacle sheath.

The commonly received opinion that the vestibule is traversed by the tentacles during their protrusion is, however, based on too many observations to be lightly dismissed.

Pergens further states (p. 506) that the median pore of *M. malusii* opens into the body-cavity. The "longitudinal muscles" mentioned by him were probably parietal muscles, while I suspect that the appearance of a "sack" between

¹ For the pore-chambers of Cheilostomata see also Waters (1898), p. 658.

the operculum and the median pore can be explained in the same way as Jullien's "cornicula" mentioned above.

Levinsen (1891, p. 285) states that the median pore, like the other pores, is closed by a membrane.

Microporella ciliata,¹ Pall.

The compensation-sac is like that of *M. malusii*, which this species further resembles in the grouped condition of the parietal muscles. I have not made out the details of the opening of the sac. There is a membranous portion in the tongue of the crescentic pore, as in *M. malusii*.

(E) Microporoid Genera.

Micropora, sp.² (figs. 57, 58).

The large lateral foramina serve for the transmission of the tendons of the depressor muscles (fig. 58, *depr.*) of the frontal membrane, as correctly stated by Jullien (1888, 4, pp. 77—81, pl. xiv, fig. 1), who terms them "opesiules." The opesiule is a complete calcareous tube (*l.r.*), the base of which joins the basal wall or the lateral wall of the zoëcium, the two conditions often occurring on opposite sides of the same zoëcium. The tube usually gives off a curved lamella (fig. 57) on its distal side, near the basal surface. From the recess thus formed originates the occlusor muscle (*occl.*), whose long tendon passes obliquely to be inserted into one of the lateral corners of the vertical flange of the operculum. The depressor muscle (*depr.*) is much stronger. Its fibres converge to a tendon which passes through the opesiule and is inserted into the frontal membrane. I regard these muscles as a special development of the parietal muscles, which are otherwise unrepresented. The calcareous frontal wall (*crypt.*) is complete, and the relations of the depressor muscles indicate that it is a cryptocyst.

¹ Hincks (1880), p. 206.

² Torres Straits, A. C. Haddon Coll.

Steganoporella alveolata, Harmer.¹

I have previously alluded² to the muscular system of this species. That of the B-zoöcia is more highly developed than in any other Polyzoon with which I am acquainted.

The B-operculum has the form shown in figs. 64, 66. Its mode of articulation differs in a striking way from that of the Cheilostomes previously considered. Although its base is continuous with the unmodified frontal membrane which stretches across the whole of the proximal half of the zoöcium, a strong basal sclerite (*b. s.*) is differentiated. Into the two ends of this are inserted, beneath the frontal membrane, two ligaments (*lig.*) which, continuing the direction of the sclerite, become attached to the strong condyles (*cond.*). The operculum is thus slung by two tight transverse cords between the two condyles, and this arrangement constitutes the hinge. The basal sclerite probably has the further object of playing over the projecting part of the structure which I have called the median process (fig. 66, *m. pr.*), and of preventing the operculum from being pulled too far by the contraction of the enormous occlusor muscles. Of these there are two pairs, which originate, with the divaricator and depressor muscles, deep down within the lateral recesses at the sides of the median process. The insertion of the occlusors is shown in fig. 64. One pair (*occl.*), which probably correspond with the normal Cheilostome occlusors, pass by strong tendons (*tend.*) into the occlusor tubercles (*occl. t.*) of the operculum, but the tendon is also continuous with a fascia (*f.*), which is connected with the strongly projecting proximal end of one of the main sclerites (*m. s.*) of the operculum. The second pair, which I distinguish as the distal occlusors (*occl.'*), are inserted into a strong transverse fascia (*f.'*) which lies between the two main sclerites. In fig. 64, which represents an operculum torn away from its zoöcium, this fascia has been displaced from its natural

¹ 1900, p. 287.

² *Loc. cit.*, p. 230.

position, which is shown in figs. 65, 66. From these it is apparent that the median part of the fascia is exactly transverse and vertical when the operculum is closed, and that on each side it curves round in order to be attached to the proximal end of the main sclerite. It thus results that while not much more than the edge of the middle part of the fascia is seen in a view from the basal or from the opposite side, the surface of the lateral parts is seen under the same conditions. I have been unable to ascertain with certainty whether the median part of the fascia is inserted into the operculum or whether it has a free edge in this region.

The origin of the muscles can best be seen in a basal view of a zoëcium from which the basal wall has been removed in the way recommended on p. 265. The lateral recesses (*l. r.*) are here seen to be almost entirely filled by four strong muscles. Of these the distal oclusors (*occl.*) are far the strongest, and they originate from the distal half of the floor of the lateral recesses. The proximal or normal oclusor (*occl.*) originates from the proximal half of the lateral recess, in its outer part. The course of its tendons (*tend.*) is indicated in figs. 64—66.

The distal oclusors, in helping to close the "orifice," probably tend to pull the whole operculum towards the distal end of the zoëcium, since their fascia is inserted largely into the projecting proximal ends of the main sclerite (figs. 65, 66). The proximal oclusors correct this tendency, as may be inferred from the direction taken by their principal tendons. When it is remembered that in this species each of the strong teeth of the operculum fits, when closed, into its own socket in the calcareous part of the zoëcium, it is obvious that the operculum must shut with great accuracy, and a proper correlation between the contractions of the two sets of oclusors no doubt has to be maintained. The accuracy of the closure is probably helped by the movement of the basal sclerite over the median process, and, of course, by the transverse ligaments uniting the operculum to the

condyles, as well as by the tension of the frontal membrane¹ continuous with the base of the operculum.

Although the distal oclusors are larger than the proximal pair, their tendency to shift the operculum from its proper position is probably adequately corrected by the proximal oclusors, which, although weaker than the others, have a more effective pull, in consequence of the greater obliquity of their tendon.

It appears to me that in certain positions assumed by the operculum, the distal oclusors would work to less advantage than the others. When the operculum is opened so far that its outer surface forms a right angle with the frontal membrane, the projecting ends of the main sclerites extend far to the distal side of the hinge. The operculum seen from its edge would have the appearance of a right-angled triangle, of which the hypotenuse is formed by the main sclerite, and a short side by the piece connecting the proximal end of the same sclerite with the base of the operculum. Both pairs of oclusors now probably pull, by means of their fasciæ inserted into the projecting ends of the main sclerite, on the short side of the triangle which is, in fact, an arm of a right-angled lever hinged at its angle. In so acting, the proximal oclusor probably pulls over the ligament (*lig.*) which connects the operculum with the condyle, the ligament thus forming a kind of pulley. The distal oclusors are probably specially important in the final stages of the closure.

Two muscles remain to be described. The depressor muscles (figs. 65, 66, *depr.*) originate from the innermost part of the lateral recess. Their tendon passes obliquely towards the proximal end of the zoëcium, ascending at the same time to reach the frontal membrane, into which it is inserted (fig. 66). Between the depressor and the proximal oclusor is a group of muscles which constitute the divaricator (*div.*).

¹ In my former paper I described this as the epitheca, a more general term which it would be better not to use in cases where the homology of the membrane with the frontal membrane of a *Membranipora* seems to be demonstrated.

At its origin this muscle is often difficult to distinguish from the depressor, but its tendon takes a different direction and is inserted into one of the ends of the basal sclerite of the operculum, or perhaps into the frontal membrane immediately adjacent to the basal sclerite.

I regard the divaricator and depressor muscles as a special differentiation of the parietal muscles of other Cheilostomes, and they are probably together the representatives of the depressor muscles of *Micropora* (figs. 57, 58), which occupy special cavities that may be compared with the lateral recesses of *Steganoporella*.¹

In the A-zoëcia the distal oclusors seem to be completely absent. The zoëcium is more like that of an ordinary Cheilostome, with a single pair of oclusor muscles.

In view of the possibility that the B-zoëcia of *Steganoporella* may represent avicularia,¹ it is worth mentioning that in the gigantic avicularia of *Flustra abyssicola* (Davis Straits) I have noticed that the oclusors are in two groups. Of these the distal group consists of an enormous mass of muscle which fills up more than the distal half of the avicularian zoëcium, and its fibres are inserted into a broad median tendon. The proximal oclusors are paired; their tendons are at a deeper level than that of the distal oclusor, and they converge towards the base of the operculum. Jullien (1888, 4, pl. xii, fig. 3) indicates the occurrence of two sets of oclusors in the avicularium of *Beania magellanica*.

V. THE PRIMARY ZOËCIUM OR ANCESTRULA.

The zoëcium which results from the metamorphosis of the larva is frequently found to show characters which differ

¹ This conclusion is confirmed by the figure given by Waters (1885, pl. xiv, fig. 42) of the mandible of *Onychocella angulosa* (? = *antiqua*), in which the formation of the hinge appears to be like that found in *Steganoporella*, while a fascia shown in the same figure closely resembles that of the distal oclusors of *Steganoporella*.

from those of the ordinary individuals of the colony. This, the primary zoëcium, has been termed the "ancestrula" by Jullien (1888, 4, pp. 27, 29), a name which he appears to have used merely to convey the idea that it was the actual ancestor ("zoëcie mère") of the other individuals of the colony. Smitt (1868, 1, p. 306) described the same thing as the "Tata form" of the Cheilostomes,¹ and clearly recognised their importance, stating that "Tata is for the Cheilostomes what Alecto showed itself to be for the Cyclostomes."

I am fully of Smitt's opinion with regard to this matter. The "ancestrula" is in all probability ancestral in the sense of retaining characters of phylogenetic importance. Smitt returns to the same subject in a later publication (1896), in which he again maintains the importance of the Tata form. Neviani (1898, p. 165) supports the same conclusion.

The frequency with which the Tata-like ancestrula (figs. 2, 6, 7) occurs in Cheilostomata is indeed remarkable, and there is a curiously small range of variation in the number of its marginal spines. I subjoin a few references to cases where an ancestrula of this type has been described.

Species.	Reference.	Number of marginal spines.	Remarks.
<i>Electra pilosa</i> , L.	Barrois, 1877, p. 241, pl. xv, figs. 5—8	5—7	
<i>Membranipora flemingii</i> , Busk	Hincks, 1880, p. 163	8—9	
<i>Membranipora craticula</i> , Alder	Hincks, 1880, p. 147	About 9	
<i>Scrupocellaria seruposa</i> , L.	Smitt, 1868, 1, p. 320, pl. xvii, fig. 42, etc.	8	Elongated.
<i>Scrupocellaria scabra</i> , Van Ben.	Smitt, 1868, 1, p. 314, pl. xvii, fig. 27	9	"
<i>Scrupocellaria reptans</i> , L.	Barrois, 1877, p. 183, pl. x, fig. 16	—	"

¹ The young zoëcia figured as *Tata rugosa* by Van Beneden ('Bull. Acad. Roy. Belgique,' xvi, pl. ii, fig. 14) are primary zoëcia of a *Membranipora*.

Species.	Reference.	Number of marginal spines.	Remarks.
<i>Menipea ternata</i> , Ell. and Sol.	Smitt, 1868, 1, p. 306, pl. xvi, fig. 15	10—11	
<i>Bicellaria ciliata</i> , L.	Smitt, 1868, 1, p. 335, pl. xviii, fig. 1	13	Infundibuliform.
<i>Bugula sabatieri</i> , Calvet	Calvet, 1900, p. 112 (fig. 13)	5	..
<i>Membraniporella nitida</i> , Johnst.	Hincks, 1880, p. 201, pl. xxvii, fig. 6	14, of which 4 are oral	
<i>Cribrilina punctata</i> , Hass.	Hincks, 1880, p. 191	12	
<i>Cribrilina monoceros</i> , Busk	Jullien, 1888, 4, pl. iii, fig. 9	9	
<i>Schizoporella spinifera</i> , Johnst.	Hincks, 1880, p. 242, fig. 13	9—10	
<i>Schizoporella unicornis</i> , Johnst.	Barrois, 1877, p. 152, pl. viii, fig. 37	8	
<i>Schizoporella crustacea</i> , Smitt	Waters, 1900, pl. viii, fig. 13	?9	
<i>Mucronella coccinea</i> , Abildg.	Waters, 1900, p. 158, pl. viii, fig. 40	13	
<i>Mucronella coccinea</i> , Abildg.	Hincks, 1880, p. 373, fig. 17	11	
<i>Mucronella peachii</i> , Johnst.	Hincks, 1880, p. 361, fig. 16	11, of which 6 are oral	Modified Tata.
<i>Mucronella variolosa</i> , Johnst.	Hincks, 1880, p. 366	10—11	? Tata.
<i>Porella concinna</i> , Busk	Smitt, 1868, 2, p. 134, pl. xxvi, figs. 109—111	7—9	Variable.
<i>Porella concinna</i> , Busk	Barrois, 1877, p. 155, pl. vii, fig. 16	8	
<i>Phylactella labrosa</i> , Busk	Hincks, 1880, p. 357	"A few"	? Tata.
<i>Microporella ciliata</i> , Pall.	Hincks, 1880, p. 207, pl. xxviii, fig. 3	9, of which 7 are oral	
<i>Microporella malusii</i> , Aud.	Hincks, 1880, pp. 211, 212, figs. 10, 11	About 7, of which 2 are oral	
<i>Microporella malusii</i> , Aud.	Neviani, 1898, p. 6, fig. 3	10	Variable.

Cases in which the ancestrula is not Tata-like:

Species.	Reference.	Remarks.
<i>Membraniporella melolontha</i> , Busk	Hincks, 1880, p. 203	Aperture membranous, but not surrounded by spines.
<i>Cribrilina annulata</i> , Fabr.	Hincks, 1880, p. 194	Like the ordinary zoœcia.
<i>Lepralia pallasiana</i> , Moll	Hincks, 1880, p. 298	Like the ordinary zoœcia.
<i>Lepralia pallasiana</i> , Moll	Barrois, 1877, p. 145, pl. vii, fig. 1	Like the ordinary zoœcia.
<i>Schizoporella</i> (<i>Hippothoa</i>) <i>hyalina</i> , L.	Barrois, 1877, p. 168, pl. ix, figs. 9—16	Like the ordinary zoœcia.
<i>Schizoporella</i> (<i>Hippothoa</i>) <i>hyalina</i> , L.	Jullien, 1888, 4, p. 29, pl. iv, figs. 1—4.	Tata-like; resembling the ordinary zoœcia; or intermediate.
<i>Schizoporella cristata</i> , Hincks	Hincks, 1880, p. 254, pl. xl, fig. 6 a	Intermediate.
<i>Smittia reticulata</i> , J. MacGill.	Hincks, 1880, p. 346	Like the ordinary zoœcia.
<i>Temachia opulenta</i> , Jullien	Jullien, 1883, p. 13 (sep.), pl. xiv, figs. 26, 29	<i>Cribrilina</i> -like.

There is no other form of ancestrula which is known to occur in a number of different types of Cheilostomes, and it is impossible not to agree with Smitt and others that the view that the primary zoœcium shows ancestral characters has much to be said for it. In the majority of described cases the ancestrula is like that shown in fig. 2, and is a typical *Membranipora*-like zoœcium. In certain forms with an infundibuliform zoœcium the ancestrula has already acquired this type. This is the case in *Bicellaria ciliata*, while in *Scrupocellaria* the ancestrula may show distinct tendencies in the same direction. It is hardly necessary to point out how thoroughly the common occurrence of a Tata-like ancestrula confirms the general conclusions arrived at in this paper. It may be remarked that this type of ancestrula is by no means unlike that of a Cyclostome (which has, however, no spines); and these Polyzoa may probably be regarded as forms in which the frontal surface remains

circular and uncalcified, while the greater part of the zoëcium is constituted by the cylindrical lateral walls.

I do not know of any Flustrine form in which the ancestrula is other than Flustrine in respect of its frontal wall. In *Membraniporella nitida* the ancestrula is Tata-like, and shows clearly that the four oral spines are of the same nature as the remaining ten marginal spines figured by Hincks. This species is particularly interesting in the fact that some of the older members of the colony produced by budding may retain the *Membranipora*-like condition (Hincks, 1880, pl. xxvii, fig. 7), and possess a series of irregular marginal spines which do not unite. The remaining zoëcia have their frontal spines arranged with the regularity characteristic of the *Cribrilinidæ* in general.

The consideration of the Cheilostomata in which the ancestrula is not Tata-like is almost as instructive as that of the first set of cases.

In *Hippothoa hyalina*, Jullien (1888, 4, p. 29) states that the characters of the ancestrula are variable. In European specimens the frontal wall is fully calcified. The same condition was found in specimens from Tierra del Fuego, although in most of the colonies from this locality the ancestrula was a circular Tata-like zoëcium, with a large membranous aperture bordered by six inwardly curving marginal spines (Jullien, pl. iv, figs. 1—3), while in a third case (*ibid.*, fig. 4) the ancestrula is at first Tata-like, but the membranous aperture subsequently calcifies, so that the orifice normal for the species is contained in a calcified oral region surrounded by the circle of marginal spines. This leads to the form described by Jullien as *Diazeuxia reticulata* (*ibid.*, fig. 5), in which the orifice of the ancestrula is completely *Schizoporelliform*.

Neviani (1898, p. 165) describes three varieties of the ancestrula of *Microporella malusii*. The first of these is an ordinary Tata form with ten marginal spines. The second more nearly resembles the normal zoëcium of this species, but agrees with one of the ancestrulæ described by Jullien (see

above) in having the orifice contained in a calcareous region which is surrounded by eight short spines. The third is particularly interesting in having a *Schizoporella*-like orifice.

Schizoporella cristata, according to the description given by Hincks, is another form with an intermediate type of ancestrula. The *Schizoporelliform* orifice is contained in a calcareous oval area, on the proximal side of which are three spines, while the orifice is provided with six oral spines.

The ordinary zoecia of the form described by Jullien as *Temachia opulenta* are lageniform, and without spines, somewhat resembling those of *Mucronella abyssicola*. The ancestrula is obviously *Cribrilina*form. I have myself observed an analogous case. A *Cribrilina* (sp.?) from Funafuti, in the collection of the British Museum, has an irregular arrangement of pores on its frontal surface, something like that found in *C. monoceros*. The primary zoecium is *Membraniporella*-like, with a perfectly regular double series of transverse bars, separated by simple slits.

Fig. 2 shows the ancestrula of a colony of *Microporella malusii*, from Naples. The frontal surface and the operculum are Flustrine. The marginal spines, some of which have been lost, were ten in number (as in one of Neviani's cases). They originate, like the frontal spines of *Cribrilina*, from foramina in the calcareous wall, a little outside the frontal membrane. The spines resemble the three oral spines with which most of the other zoecia of the same colony are provided.

An ancestrula of *M. ciliata*, also from Naples, is somewhat similar; but it differs in having a distinct "area," or calcification of the proximal part of the frontal surface. The living tissues have disappeared, and I am, unfortunately, unable to state certainly whether the calcified area is a cryptocyst or not. This is, however, indicated by the way in which it slopes down steeply from its periphery, so that its inner edge lies at a deeper level than its outer margin. There are ten long marginal spines.

One or two colonies of *M. impressa* (S. Devon) are particularly interesting in having a Schizoporelliform ancestrula.

In *Schizoporella vulgaris* (Naples) I have found an ancestrula (fig. 6), like that of *Microporella ciliata*, with ten marginal spines and a calcified plate (*crypt.*) which is probably a cryptocyst. This resembles the cases which I have described as "intermediate" between the typical Tata form (fig. 2) and the adult condition with a completely calcified frontal wall. It appears to me that these "intermediate" Tata forms deserve special attention. It remains to be seen whether the calcified plate internal to the circlet of spines is really a cryptocyst or not. Taking into consideration the frequency of the occurrence of a cryptocyst in the *Amphiblestrum* group of *Membranipora* (although the morphology of this part has yet to be determined in many of these cases) and in such a typical *Membranipora* as *M. delicatula*, Busk (e. g.)—and noticing, moreover, the presumed occurrence of a cryptocyst in *Bicellaria grandis* (fig. 1),—the presumption appears to be that the plate marked *crypt.* in fig. 6 is really a cryptocyst. The bearing of these cases on the phylogeny of the Cheilostomata is considered in a later part of this paper (p. 334).

Cribrilina radiata,¹ Moll.—The study of a young colony of this species has led me to altogether unexpected conclusions, which probably indicate that this species is not really a *Cribrilina* at all. The ancestrula (fig. 7) is a Tata form with eleven marginal spines, of which the proximal (*sc.*) is broad and somewhat scutum-like, lying horizontally over the frontal membrane, while the others are erect or somewhat reclined outwards. Each of the erect spines bears a minute calcareous, denticulated lobe which originates about halfway up the spine, and lies horizontally, directed inwards over the frontal membrane. The outline of the ancestrula is somewhat concealed by the basal lobes of the younger zoëcia.

Two zoëcia in contact with the ancestrula are smaller than the rest, and are doubtless those which were first formed by

¹ Hincks, 'Brit. Mar. Pol.,' 1880, p. 185.

budding. One of these (*z.*') is on the distal side of the ancestrula, and the other (not drawn) is on its apparent left side. On each of the five oral spines of these zoöcia is a minute calcareous lobe resembling those of the marginal spines of the ancestrula. The lobes may be indicated by still smaller projections on the oral spines of the other zoöcia.

In all the ordinary individuals there occur, on the proximal side of the operculum, a pair of delicate, membranous, elongated structures (*sp.*), which are described by Hincks (1880, p. 186) as vibraculoid setæ. Smitt¹ ascribes to them a sensory function.

The structures in question have no claim to be regarded as vibracula. There is no trace of any muscles connected with them, and there can be no doubt that they continue the line of the oral spines. This line is, in reality, prolonged all round the zoöcium, the succeeding spines (*sp.*') being delicate membranous papillæ which barely project above the calcareous surface, and are obviously in series with the oral spines and the "vibraculoid setæ." The base of each papilla is a pore in the calcareous frontal wall. Comparison with the ancestrula and with the calcareous lobes of the oral spines of the younger zoöcia seems to indicate that the membranous papillæ, together with the oral spines, correspond with the marginal spines of the ancestrula. The calcareous lobes of that individual are, in consequence, the representatives of the bars which have united to form the frontal wall of the ordinary zoöcia, while the scutum-like spine of the ancestrula is probably the equivalent of one of the denticulated lobes on the other spines.

It has usually been assumed that the radiating series of pores correspond with the slits between the bars. This is certainly the case in *C. philomela* (fig. 8); but in *C. radiata* (fig. 7) there can be no doubt that the pores are in the same radii as the membranous marginal spines. I can

¹ "Floridan Bryozoa," II, 'K. Svenska Vet.-Ak. Handl.,' xi, 1873, No. 4, p. 22.

find no indication of a double calcareous wall in this species, and it therefore appears to me that the frontal wall is in a Lepralioid condition, the inner calcareous wall alone being developed, and being perforated by a series of vacuities, while the outer wall remains membranous. The probability that a layer of living tissue (epitheca) overlies the calcareous wall is increased by the fact that the surface of the latter is ridged, a condition which is usually associated with a covering of living tissue.

The lateral junctions of the frontal bars are indicated by prominent radial ridges, each of which rises to a small tubercle just inside the line of the membranous papillæ above described. The pores consequently lie, as described by other observers, in radiating furrows. That the union between the bars has not been complete is indicated by the fact that a thin line of air in some cases underlies the ridge.

The consideration of these facts suggests that the frontal wall has here not been formed by the overarching of the main spines, but by the development of the inner lobes of branched spines. It is impossible not to be reminded of the condition described by Jullien (1886, p. 609) in the Cretaceous Steginoporidæ, where a calcareous wall¹ is formed by the growth of branched peristomial spines.

I have, unfortunately, been unable to make out the condition of the compensation-sac in *C. radiata*, but it is well known that this species may possess a well-marked median pore (*m. p.*). In addition to this Microporelloid feature, it may be noticed that the zoëcium is surrounded by a flat basal lobe formed of pore-chambers,² as in *Microporella ciliata* and *M. malusii*. These are not present in *C. philomela*.

There can, I think, be little doubt that *C. radiata* cannot be retained in the same genus with *C. philomela*, and it is possible that its affinities are rather with the Microporellidæ.

Cribrilina figularis, Johnst. (Plymouth), is another species in which, so far as can be judged from dry material,

¹ Which, however, would not appear to correspond with that of *C. radiata*.

² The details of the pore-chambers are not indicated in fig. 7.

there is evidence that the frontal shield is formed by a series of horizontal lobes developed from the main spines. These latter are indicated with great distinctness as a curved series of conical calcareous papillæ, the truncated end of which is a membranous fenestra, and stands up at a higher level than the frontal shield, which is encircled by the papillæ. The radiating series of pores here correspond with the intervals between the spines, as in *C. philomela*.

The existence of great differences between the opercula of different species at present referred to *Cribrilina* suggests that the genus is an unnatural one, representing a stage in the evolution of the Lepralioid zoëcium, which has been arrived at independently in several cases, and is merely the result of incomplete lateral fusion of the bars which compose the frontal shield.

VI. CLASSIFICATION OF THE CHEILOSTOMATA.

While recognising the danger of drawing taxonomic conclusions from the study of a single organ, I cannot but think (with Jullien) that the consideration of the frontal surface is capable of settling some questions of this kind in the Cheilostomata. It would, I think, be premature to propose a new classification, but the observations above described suggest certain lines along which the existing classifications may be amended. I confine myself to groups to which I have paid some attention, leaving entirely out of consideration a large proportion of recent genera.

(1) The Membraniporidæ and Flustridæ are indicated as the most primitive group of Cheilostomes by the relations of their frontal membrane and parietal muscles, and by the evidence afforded by the primary zoëcium of many other Cheilostomes. For this group I think that Smitt's name *Flustrina* should be retained. It remains to be seen whether many of the *Amphiblestrum*-like forms of *Membranipora*, in which a calcareous cryptocyst (?) is developed, are more nearly related to this group or to the Microporoid series.

In many of the Flustrina there is an elongated frontal membrane which is typically depressed by a series of parietal muscles. A frontal membrane of this description occurs in the Membraniporidæ (part), Flustridæ, and Farciminariidæ, and in such genera as *Bugula* and *Beania*.

In other Flustrine forms (*Dimetopia*, *Bicellaria*) the zoëcium becomes elongated and usually infundibuliform, with a concurrent reduction in the size of the frontal membrane—a tendency which may be shown even in the ancestral. The reduction of the frontal surface may be correlated with a reduced number of parietal muscles.

(2) Another series seems to be constituted by *Scrupocellaria*, *Menipea*, and *Caberea*, a group of "Cellularine" forms in which certain species in each genus have the frontal membrane protected by the excessive development of a single lateral spine, the fornix or scutum, which may, however, be vestigial or absent. In some cases, as in *Menipea jeffreysii*,¹ Norman, the scutum is so largely developed that a complete calcareous frontal wall appears to exist. Round the edge of this is a slit which is the interval between the edge of the oval scutum and the calcareous margin of the frontal membrane. The scutum originates from a broad calcareous base just proximal to the operculum, and on its inner side. It has a nearly straight suboral edge, which meets the basal sclerite of the approximately semicircular closed operculum. On the outer side the scutum locks firmly into a strong tubercle developed from the edge of the zoëcium. If the scutum were to fuse completely with the edge of the frontal surface a compensation-sac would be produced which would have had a different phylogenetic history from that of many other Cheilostomes. I see no reason why cases of this kind should not exist,² and it is possible that evidence to that effect might be forthcoming in

¹ Norman, 'Ann. Mag. Nat. Hist.' (6), xii, 1893, p. 446.

² The figure given by Waters (1888, pl. i, fig. 1) suggests that this might be the case in *Catenaria bicornis*; and the structure of *C. lafontii* (fig. 49) is not irreconcilable with this view.

the existence of a single communication between the living tissue of the frontal wall and that of the body-cavity, corresponding with the base of the scutum.

The group is usually characterised by the high development of avicularia and vibracula.

(3) The Microporoid series includes Micropora, Thalamporella, Diploporella,¹ and Steganoporella, all characterised by the development of a cryptocyst² or calcareous lamella which grows beneath the frontal membrane. In the forms which have been examined, the parietal muscles are reduced to a single pair of highly developed depressor muscles which commonly traverse opesiules³ to reach the frontal membrane; or, in Steganoporella, to a pair of depressor muscles and a pair of divaricators. The series probably includes a large number of the Cretaceous forms allied to Onychocella, as well as the recent *O. abyssicola*, Smitt, etc. Some of the recent species sometimes placed in the Membraniporidæ may also belong to this group.

(4) The Cribrilinidæ, including Membraniporella and Cribrilina (part), are forms in which a calcareous frontal wall is developed, ontogenetically, by the overarching of a series of marginal calcareous spines. This process has been observed by various authors in a certain number of species. I have shown above that *C. radiata* differs in important respects from the typical Cribrilinidæ.

(5) In *Umbonula verrucosa* the frontal shield originates ontogenetically as a calcareous lamina, perforated by a conspicuous series of marginal pores, which grows over the Flustrine frontal membrane, leaving a wide space between itself and that membrane. The parietal muscles develop in the situations which they will occupy in the mature zoecium. I consider that the marginal pores correspond with the origins of as many Cribrilina-like spines which have united to form the frontal shield. The external calcareous

¹ MacGillivray (1887), p. 207.

² Cf. Harmer (1900), p. 228.

³ Ibid., p. 230.

layer of the spines is now represented only by a membranous epitheca, composed of living tissue bounded by an external cuticle, while the frontal shield itself corresponds with the deeper layer of the original series of spines (fig. 12). The frontal shield extends to the sides of the operculum as a pair of calcareous shoulders, which commonly bear avicularia, while the suboral part of the shield may support a median avicularium which guards the entrance to the incipient compensation-sac. "*Mucronella*" *pavonella*, Alder (p. 296), probably belongs to *Umbonula*, which may also include *M. bicuspis*, Hincks.¹

So far as I can judge from dry preparations, the development of the frontal shield takes place in the *Umbonuloid* manner in *Porella compressa*, *P. saccata*² (= *P. elegantula*, auctt.), *P. (Palmicellaria) skenei*, *Mucronella coccinea*, and *Escharoides sarsii*. In specimens of *U. verrucosa* from Naples³ the suboral avicularium has a mandible which closely resembles that described by Waters⁴ in species of *Porella*, being of more or less semicircular form, with denticulated margin and conspicuous Λ -shaped main sclerite. This confirms the association of *Porella* with *Umbonula*. I am inclined to think that the presence of marginal areolæ (as shown in figs. 10, 11) is of great importance as indicating affinities in this direction.

(6) In the *Lepralioid* type of calcification (figs. 13—15, 41) the young zoecium frequently has a *Flustrine* appearance; but the frontal shield seems to result from a direct calcification of the frontal membrane. The compensation-sac is developed, after calcification is complete, as an invagination formed at the base of the operculum. This is the case in *Lepralia*, *Schizoporella*, *Urceolipora nana*, *Euthyris*, *Euthyroides*, *Vittaticella*, and *Catenicella*. The

¹ 'Ann. Mag. Nat. Hist.' (5), xi, p. 201.

² Waters, 1900, p. 81.

³ It is, however, possible that this form is not identical with the littoral form common on the south coast of England, which I have alluded to as *U. verrucosa*.

⁴ 1900, pl. xi.

parietal muscles at first radiate to the base of the operculum (figs. 13, 14), but their insertions become shifted by the growth of the compensation-sac, until in the fully formed zoëcium (figs. 33, 46) they have much the same relations as those of *Umbonula* (fig. 11) or of *Cribrilina philomela* (fig. 9). It seems difficult to avoid the conclusion that the floor of the compensation-sac represents in all cases the original frontal membrane, as indicated in the diagrammatic figs. 43, 44, 50. From this it would appear to follow that in the Lepralioid mode of development the phylogenetic history of the compensation-sac implied by those figures has been modified ontogenetically, in such a way that the apparent frontal membrane of the young zoëcium is in reality an epitheca (cf. fig. 12, *ep.*), that the calcareous frontal shield is next formed beneath the epitheca, and that the frontal membrane only makes its appearance when the compensation-sac is invaginated. The fully developed frontal wall of a *Lepralia* would, on this view, be represented by a completely fused series of *Cribrilina* spines, of which the outer layer is membranous (= epitheca) and the inner layer is calcareous (fig. 50); the marginal pores on this view corresponding with the original communications between the *Cribrilina* frontal bars and the general body-cavity.

Another view is, however, possible. The membranous covering of the young zoëcium may be, as it appears to be, the frontal membrane, and the frontal shield may be a cryptocyst which grows beneath the membrane towards the distal end of the zoëcium.

Thus in fig. 34 the calcareous frontal wall from which the papilla (*calc. p.*) is seen to arise would be the cryptocyst, which has grown distally beneath the frontal membrane (*ep.*). The invagination of the compensation-sac would then represent an ingrowth of a fold of the frontal membrane round the free distal border of the cryptocyst. The actual ontogeny of the organ would then agree completely with its supposed phylogeny in such cases as *Lepralia*, *Schizoporella*, and *Euthyris*. The Microporoid series of genera would thus

acquire great importance in throwing light on the structure of some of the Escharine forms. If a given species has a Cribrilininid ancestry, it might be expected that the roof of the compensation-sac would be formed by the deeper surface of the frontal shield (figs. 12, 50). If, on the contrary, the frontal shield is a cryptocyst, it might be anticipated that the compensation-sac would have a membranous roof distinct from the frontal shield. Although this arrangement is indicated in fig. 34 (*Euthyris oblecta*) I cannot assert positively that it is the case in that species. There is, however, no doubt of the existence of a complete membranous roof to the compensation-sac in *Euthyris clathrata* (figs. 26, 27).

There are other considerations which indicate that the frontal shield may, in certain Escharina, be a cryptocyst. Numerous Membraniporidae, both recent and fossil, show a tendency to develop a cryptocyst, although in many cases complete proof that the calcified "area" is of this nature is not yet forthcoming. The case of *Bicellaria grandis* (fig. 1) is again uncertain. But the fact which is, perhaps, most significant is that in certain species of *Schizoporella* (fig. 6), *Microporella*, etc., the region encircled by the marginal spines of the primary zoecium is partially calcified proximally. It appears to me to be of great importance to ascertain whether this calcified portion is of the nature of a cryptocyst or not;—in other words, whether the frontal membrane extends as far as the sharp ridge on the inner side of the base of the spines. I can hardly doubt that this is the case; and if so, the Microporoid origin of Escharine forms in which the compensation-sac develops as an invagination at the base of the operculum would appear to be indicated. The Cribrilina-like character¹ of certain species of *Catenicella*, in which the sac develops in this way, is, however, a difficulty which requires further explanation.

¹ This character is indicated in fig. 55 of the present paper, and in many figures on pls. i and ii of MacGillivray's "Monograph of the Tertiary Polyzoa of Victoria" ('Trans. Roy. Soc. Vict.,' iv, 1895). The Escharine nature of the Catenicellidae is noticed by Smitt (1868, 2, p. 46).

It may perhaps indicate that the cryptocyst may have co-existed with a Cribrilina-like frontal shield, and it is not indeed impossible that the plate *pl.* in fig. 54 may represent the cryptocyst.

Calvet (1900, pp. 163, 436), who gives an excellent account of the structure of many marine Ectoprocta, has already expressed the opinion that in Lepralioid forms the calcified frontal wall is a cryptocyst. He states that while in most species the cryptocyst and the more external "ectocyst" (= epitheca) occur only on the frontal wall, in *Retepora* the whole external surface is two-layered. This and the analogous case of *Urceolipora nana* require further study.

Calvet does not recognise the compensation-sac, although it is indicated clearly in his pl. viii, fig. 9 (*Catenaria lafontii*), and its development is partly described on p. 399 and figured in pl. xiii, fig. 20 (*Lepralia foliacea*). The account which he gives (p. 168) of the median pore of *Microporella* does not agree with my results. I think that the discrepancies are easily to be explained by the fact that in highly calcified species the compensation-sac is so delicate that it may easily be overlooked in sections which have been distorted by decalcification and embedding in paraffin.

Calvet, after quoting in extenso (p. 276) Jullien's very accurate account of the mode of articulation of the operculum and its relations to the compensation-sac, proceeds to give it a complete denial (p. 278). He explains the existence of the parietal muscles in species with a calcified frontal wall by supposing that wall to have an amount of flexibility sufficient to allow itself to be depressed by the parietal muscles. The fact that these muscles are not inserted into the calcified wall is a sufficient answer to this suggestion.

Euthyris may be regarded as a highly modified Escharine form, which has acquired a *Flustra*-like habit. The irregularity of the bars of *E. clathrata*, and the fact that they are entirely beneath the epitheca, suggest that they do not represent the tubular frontal bars of *Cribrilina*, but are due to deficiencies in the calcification of

what was once a completely calcified wall. The epithecal investment is particularly obvious in *Euthyris*. It is possible that the basal epitheca is a reminiscence of a time when the frond was composed of two layers of zoëcia, placed back to back. I have previously (1901, p. 17) pointed out that there is evidence that many Cretaceous Cheilostomes were provided with a similar basal epitheca.

(7) In the Microporelloid series the compensation-sac opens by a "median pore," which has probably resulted from the closure of a Schizoporelliform sinus. This view, which has been adopted by Gregory¹ as the basis of his division Schizothyriata, receives support from the occasional occurrence in the group of an ancestrula with a Schizoporella-like operculum (cf. p. 324). Gregory's classification places Schizoporella with Microporella, and separates both from Lepralia. It appears to me, on the contrary, that Lepralia and Schizoporella cannot be separated from one another, whereas Microporella is a distinct step in advance of the other two.

This section includes Microporella, and perhaps "*Cribrilina*" radiata among encrusting forms; while *Calwellia* (in which I place *Urceolipora dentata*), *Ichthyaria*, and *Onchoporella* also belong to it. I think Miss Jelly² goes too far in placing *Siphonicytara* in *Calwellia*. *Onchoporella* may include *O. bombycina*, auctt., *O. ligulata*, Busk,³ and *O. selenoides*, Ortmann,⁴ but there seems to be little except its habit to distinguish it from *Calwellia*. The suture which in the latter genus connects the middle line of the operculum with the median pore probably indicates the

¹ 1893, p. 224.

² 1889, p. 33.

³ 'Quart. Journ. Mic. Sci.,' viii, 1860, p. 282. This species has a close resemblance to *O. bombycina*, auctt. It was clearly by an oversight that Busk (1884, p. 104, n.) referred *Scruparia diaphana* instead of *Carbasea ligulata* to his genus *Onchoporella*; the original figures of the two species having appeared on the same plate of this Journal.

⁴ 'Arch. f. Naturg.,' lvi (i), 1890, p. 28.

incomplete fusion of two suboral calcareous spines by which the median pore has been cut off from the orifice. The characteristic infundibuliform pores on either side of the suture are perhaps parts of these spines.

It may be pointed out in conclusion that the principal results of this paper appear to be completely in accordance with palæontological evidence. In Canu's important memoir on Cretaceous Cheilostomata (1900), the rarity of the Cretaceous Monodermata, a group instituted by Jullien, which corresponds in the main with the *Escharina* of other authors, is pointed out (p. 458), and only three pages are devoted to their consideration. Nearly all the remaining species belong to the groups designated as *Flustridæ*, *Onychocellidæ*, *Opesiulidæ*, and *Costulidæ*. The *Flustridæ* correspond with the *Flustrina* of other authors. The *Onychocellidæ* are an important series, still represented by the recent genus *Onychocella*, characterised by the great development of the cryptocyst, and usually by the presence of a peculiar form of vicarious avicularium known as an onychocellium. The *Opesiulidæ* include the recent *Microporidæ* and *Steganoporellidæ*. The *Costulidæ* are equivalent to the *Cribrilinidæ*. These four groups are all represented by a large number of genera and species.

Gregory (1896, preface) states that "it is among the Jurassic deposits that we have to seek the ancestors of existing types of Bryozoa." His Catalogue of Jurassic forms includes only two Cheilostomes, one of which is a *Membranipora* and the other an *Onychocella*.

It thus follows that the Jurassic and Cretaceous Cheilostomes belonged, for the most part, to the *Diplodermata* of Jullien (= *Athyriata*, Gregory, 1893, p. 223). Both Jullien, in a memoir (1888, 4, p. 8) published subsequently to the original introduction of his term *Diplodermata*, and Gregory include the *Cribrilinidæ* in the sub-orders respectively adopted by them. It appears to me that the *Cribrilinidæ* would be better left out of the sub-order. They do not agree with Jullien's definition of the *Diplodermata* as forms with a

double "ectocyst;" that is, as forms in which there is a calcareous cryptocyst internal to the frontal membrane. Although Gregory was justified in regarding the Cribrilinidæ as forms in which the frontal membrane persists, the view which I take of the compensation-sac involves the conclusion that the membrane persists even in the Escharine genera. The Cribrilinidæ may be regarded as a transition-group between the Flustrine and the Escharine forms; but, as I have pointed out above, it appears to me that the family, as at present constituted, is not a natural one.

Although it is very difficult to draw a line between the more simple species of *Membranipora* and those in which a definite cryptocyst occurs, it is hardly possible to speak of some of the former as diplodermatous. Although the term *Athyriata* is antedated by *Diplodermata*, it is more in accordance with the structure of recent genera than Jullien's term, and in the present state of our knowledge it would perhaps be well to accept it as the name of a group including those forms in which the frontal membrane persists in more or less its primitive form, whether a cryptocyst is present or not below it.

The *Athyriata* are thus, on palæontological evidence, the oldest group of Cheilostomes. In the Cretaceous period they had already differentiated themselves into the types of structure represented by *Membranipora*, *Onychocella*, and *Micropora*, in the latter two of which the cryptocyst is well developed. The Cretaceous period is also characterised by the occurrence of a number of species belonging to the transitional Cribrilinid type. So far as the evidence goes at present, the true Escharine forms were then much inferior in number to the dominant *Athyriate* group.

With regard to the other parts of Gregory's classification (1893, p. 223), it appears to me that the sub-order *Stolonata* includes several families of very different affinities, the *Eucrateidæ* at any rate probably belonging to the *Athyriata*. Some of the *Cellularina* are also *Athyriate*, while I agree

with Gregory (p. 222) that *Catenicella*, which he places nevertheless in the Cellularina, has but little to do with the other Cellularines. I cannot accept the divisions Schizothyriata (forms with a sinus or a median pore) and Holothyriata (other Escharine genera), because it seems to me that there is no essential difference between the Schizothyriate *Schizoporella* and the Holothyriate *Lepralia*. Both these genera probably require a good deal of rearrangement and subdivision; but among recent species there are certainly cases in which it is an open question to which genus they should be referred. It would perhaps have been better to institute a new sub-order for the Microporelloid genera. The question is complicated by the fact that all "median" pores are not homologous structures. It is a well-known fact, for instance, that in some cases (e. g. *Adeonella*) the median pore is simply a peristomial opening.

VII. SUMMARY OF THE MORE IMPORTANT RESULTS.

(1) A large number of Cheilostomata are provided with a "compensation-sac," a structure described by Jullien, which opens at the proximal border of the operculum or by a "median pore." Muscles which usually originate from the lateral walls of the zoecium are inserted into the floor of the compensation-sac, which they dilate by their contraction, thereby helping to force out the polypide. The constant change of water in the compensation-sac probably has a respiratory importance. The sac is frequently found in zoecia whose polypides have undergone histolysis.

(2) The floor of the compensation-sac corresponds in whole or in part with the membranous frontal surface (for which the name "frontal membrane" is suggested) of a Membranipora; and its muscles are homologous with the parietal muscles of the same genus. The compensation-sac appears to have been evolved in more than one way.

(3) In Cribrilinidæ it is well known that a calcareous wall (for which and for other calcareous frontal walls the name

"frontal shield" is suggested) is developed as a series of marginal spines, which overarch the frontal membrane. This leads to the condition found in some Escharine forms, in which, as in *Umbonula*, the calcareous frontal shield similarly overarches the frontal membrane.

(4) The frontal shield of the Microporidæ and Steganoporellidæ is of the nature of a cryptocyst,¹—that is to say, of a calcareous lamella, which grows horizontally across the body-cavity beneath the frontal membrane. This has, perhaps, led to the condition found in *Lepralia*, *Schizoporella*, and other genera, in which the compensation-sac is developed as an invagination at the base of the operculum and passes to the deeper side of the frontal shield, which is on this view a cryptocyst.

(5) The epitheca, or layer of living tissue, bounded by a cuticle, which covers the frontal shield, may represent the entire frontal membrane (Microporidæ, etc.), or only a part of that membrane (*Lepralia*, etc.), or it may have been derived from the outer calcareous layer of the frontal bars of a *Cribrilina*-like form (*Umbonula*). The epitheca is frequently responsible for the addition of secondary calcareous matter to the frontal shield, and in some cases (*Cellepora*, etc.) for the formation of new generations of zoœcia which are superposed on the older ones.

(6) The operculum, in its more primitive condition, is merely part of the frontal membrane strengthened by a semicircular marginal flange. It acquires a firmer texture and a more elaborate arrangement of its thickened parts as the result of its articulation with calcareous portions of the zoœcium. While ocluser muscles may be regarded as an essential adjunct of the operculum, definite divaricators seem to have been evolved as a modification of the distal pair of parietal muscles.

(7) The consideration of the relations of the frontal surface involves considerable rearrangements in the Cheilostomata (see section on classification, p. 329).

¹ Cf. Harmer (1900), p. 228.

(8) The results of the study of the compensation-sac and frontal surface generally are confirmed by an examination of the primary zoëcium or "ancestrula" (Jullien).

(9) Two new species (*Euthyris clathrata* and *Lepralia haddoni*) are described, and a new genus (*Euthyroides*) is suggested for *Euthyris episcopalis*, Busk.

(10) The operculum of *E. clathrata* is of a specially complicated type. The vestibule, or first part of the introvert, is provided with a chitinous distal lip, for which the name "labium" is suggested.

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¹ Many of the references are given as foot-notes.

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EXPLANATION OF PLATES 15—18,

Illustrating Mr. Sidney F. Harmer's paper “On the Morphology of the Cheilostomata.”

REFERENCE LETTERS.

av. Marginal areolæ. *avic.* Avicularium. *b.* Polypide-bud. *b.b.* Brown body. *b.ep.* Basal epitheca. *butlr.* Buttress of operculum. *cæ.* Cæcum of stomach. *calc.p.* Calcareous papillæ. *cond.* Condyle, or chitin surrounding the decalcified condyle. *c.p.* Communication pore. *c.s.* Compensation-sac. *crypt.* Cryptocyst. *depr.* Depressor muscle of frontal membrane. *div.* Divaricator muscle of operculum. *d.w.* Distal wall of zoecium. *ep.* Epitheca. *ep.c.* Cavity beneath epitheca. *f.* Membranous fenestra. *f.b.* Frontal bars or costules. *fl.* Vertical flange of operculum. *f.m.* Frontal membrane. *f.sh.* Frontal shield. *f.sh.d.* Distal lobes of frontal shield. *inf. avic.* Infra-avicularian compartment. *j.* Chitinous joint. *lb.* Labium. *l.r.* Lateral recess. *m.c.* Marginal cavity of frond. *m.p.* Median pore. *m.t.* Median tooth (lyrula) of *Smittia*. *occl.* Ocluser muscles of operculum. *op.* Operculum. *op.gl.* Opercular glands. *ov.* Ovicell. *p.* Pores. *p.c.* Pore-chambers. *p.d.* Parieto-diaphragmatic muscles. *p.m.* Parietal muscles. *p.m.'* Distal group of parietal muscles. *p.v.m., p.v.m.'* Parieto-vaginal muscles and bands. *p.w.* Proximal wall of zoecium. *p.z.* Primary zoecium. *r.m.* Retractor muscles of polypide. *s., s.'* Suture in calcareous wall. *scl.* Chitinous sclerite. *sp.* Spine. *sup. avic.* Supra-avicularian compartment. *t.* Tentacles. *t.s.* Tentacle sheath. *v.* Vitta. *vest.* Vestibule (= diaphragm). *z.'* Daughter-zoecium.

Fig. 12 (Pl. 15) and Figs. 43, 44, 50, and 51 (Pl. 17) are diagrammatic representations of the points respectively illustrated by them. Fig. 18 (Pl. 16) is $\times 1$. Figs. 19, 20, 22, 32, 35, and 36 (Pl. 16) were drawn to the same scale (camera lucida, Zeiss, A obj.). Fig. 19 was not reduced, but the others were reduced $\frac{1}{2}$. All the remaining figures are more highly magnified (Zeiss, C obj.; afterwards $\times \frac{1}{2}$).

The specimens figured belong to the Museum of Zoology at Cambridge.

PLATE 15.

FIG. 1.—*Bicellaria grandis*, Busk, var. *producta*, MacGill. Showing the frontal membrane (*f. m.*), into which a single pair of parietal muscles (*p. m.*) are inserted; the plate *crypt.* is probably a cryptocyst. The zoëcium has given rise by budding to two younger zoëcia (*z.*'), and is connected with its lateral neighbour by the communication pore (*c. p.*). Each of the three communication pores is surrounded by a strong calcareous ring; *occl.*, ocluser muscles.—Victoria.

FIG. 2.—*Microporella malusii*, Aud. Primary zoëcium or ancestrula. The frontal membrane is typically Flustrine, its calcareous margin bearing ten spines, of which three are oral spines.—Naples.

FIG. 3.—*Flustra pisciformis*, Busk. Distal view of operculum, showing the vertical flange *fl.*—Bass's Strait (Challenger Collection).

FIG. 4.—*F. pisciformis*. An entire zoëcium from the same slide. The distal groups of parietal muscles (*p. m.*') probably act as divaricators of the operculum.

FIG. 5.—*Dimetopia spicata*, Busk. The funnel-shaped zoëcium is closed by a terminal frontal membrane which is depressed by a single pair of parietal muscles (*p. m.*).—Victoria.

FIG. 6.—*Schizoporella vulgaris*, Moll. Primary zoëcium. The region inside the marginal spines is partly occupied by a calcareous plate (*crypt.*), which is probably a cryptocyst.—Naples.

FIG. 7.—*Cribrilina radiata*, Moll. Primary zoëcium (*p. z.*) and three younger zoëcia. The Flustrine frontal membrane of *p. z.* is overarched by a proximal scutum-like spine (*sc.*), while the ten other spines bear minute horizontal calcareous lobes which project from their inner sides. The oral spines of the zoëcium *z.*' bear similar lobes. In all except the primary zoëcium the series of oral spines is continued by the pair of elongated membranous spines (*sp.*) on either side of the median pore (*m. p.*), and further by the small papillæ (*sp.*'), which correspond with the marginal spines of the primary zoëcium. The series of pores are in the same radii as these modified spines. The pore-chambers are not indicated.—Naples.

FIG. 8.—*Cribrilina philomela*, Busk. The frontal shield is composed of hollow calcareous bars (*f. b.*), alternately arranged on the two sides of the zoëcium. The rows of pores correspond with the intervals between the bars. The compensation-sac (*c. s.*) and some of the parietal muscles (*p. m.*) are indicated.—Marion Is. (Challenger Collection).

FIG. 9.—Basal view of another zoëcium from the same colony. The distal parietal muscles (*p. m.*') are stronger than the other groups (*p. m.*).

FIG. 10.—*Umbonula pavonella*, Alder. A series of large areolæ¹ occurs

¹ Cf. Gregory (1893), p. 221.

round the proximal and lateral borders of the frontal shield, which extends as two wing-like processes (*f. sh. d.*) along the borders of the orifice. Each of these processes bears a lateral oral avicularium (*avic.*). The calcareous floor of each areola is perforated by a pore through which a strand of living tissue (*x*) passes to join a sheet of similar tissue which overlies the frontal shield. The frontal membrane lies at a much lower level than the frontal shield (cf. Fig. 12), and the orifice is not represented.—North Sea.

FIG. 11.—*Umbonua verrucosa*, Esper. Young zoëcium. The frontal shield is represented by a crescentic calcareous film, already divided by radiating buttresses on its upper surface into distinct areolæ (*ar.*), in the floor of each of which is a pore (*p.*). The distal wings (*f. sh. d.*) of the shield are distinctly indicated. The parietal muscles (*p. m.*) are seen through the calcareous film.—Plymouth.

FIG. 12.—Diagrammatic longitudinal bisection of an Umbonuloid Cheilostome.

FIGS. 13—17.—*Euthyroides episcopalis*, Busk.—Victoria.

FIG. 13.—Young zoëcium. The limit of calcification is the line *x*, distal to which is an accumulation of nuclei (*c. s.*), to which the parietal muscles (*p. m.*) radiate. The distal wall (*d. w.*) of the zoëcium is not completely calcified; *e.*, growing edge of zoarium.

FIG. 14.—An older but much shorter zoëcium, in which calcification is nearly complete. The median pore is becoming delimited by the simultaneous growth of the median tongue (*tg.*) of calcareous matter and the lateral processes (*l. p.*). The parietal muscles (*p. m.*) radiate towards a mass of tissue at the base of the operculum; *c. p.*, communication pores.

FIG. 15.—Older zoëcium, with completely calcified walls. The compensation-sac (*c. s.*) now possesses a distinct cavity, but is still of small extent.

FIG. 16.—Mature ovicell, borne by a fertile zoëcium which has an ordinary zoëcium on its proximal side.

FIG. 17.—A fertile zoëcium with a young ovicell (*ov.*) represented by a concave plate, which will constitute the inner wall (*i. w.*) of the ovicell, and by a second plate, which will form the outer wall (*o. w.*). The Cribrilina-like frontal bars (*f. b.*) differ from those shown in Fig. 16 in correlation with the presence of an ovicell (*ov.*) on the proximal side of this zoëcium. On the distal side of the young ovicell (*ov.*) is a still younger fertile zoëcium whose frontal bars are only half developed.

PLATE 16.

FIGS. 18—31.—*Euthyris clathrata*, n. sp.—Port Jackson.

FIG. 18.—Ends of several branches, $\times 1$.

Fig. 19.—Operculum and labium (*l.*) from a dry specimen. The arrow indicates the entrance to the compensation-sac.

Fig. 20.—Part of the frontal surface of a branch; *m.*, lateral margin of the frond, the epitheca of which covers a continuous marginal cavity (*m. c.*), strengthened by calcareous bars (*c. b.*) in its frontal wall. Two of the opercula (*B, B'*) are different from the others (*A*).

Fig. 21.—The zoëcium *B* and parts of its neighbours, of the preceding figure; *d. w.*, distal wall of *B*; *p. w.*, proximal wall of its distal neighbour. (For *x*, see Fig. 30.)

Fig. 22.—Basal view of several zoëcia; *m.*, mesentery-like lamella of chitin connecting the proximal part of the basal wall of the zoëcium with the basal epitheca.

Fig. 23.—Labium, seen from the distal side.

Fig. 24.—Open operculum (*op.*) and labium (*lb.*), in side view; *cond.*, chitinous lamella covering the condyle (decalcified); *scl.*, sclerite surrounding the cavity in which the operculum lies; *c. s.*, beginning of floor of compensation-sac; *vest.*, entrance to vestibule.

Fig. 25.—Closed operculum and labium; *occl.*, ocluser muscles; *div.*, divaricator muscles. (For *x*, see Fig. 30.)

Fig. 26.—Longitudinal section (thick) of a zoëcium; *b. ep.*, basal epitheca; *ep. c.*, cavity beneath epitheca; *f. b.*, a bar belonging to the frontal shield.

Fig. 27.—Distal part of the operculum, with the labium (*lb.*), seen in a thick transverse section of the zoëcium (not decalcified). The condyles (*cond.*) are in the foreground, and the part of the operculum immediately connected with them (cf. Fig. 29) is not indicated. The labium and the biting edge of the operculum are seen at a much deeper focus; *p.*, pores.

FIGS. 28—31.—Transverse sections of opercula.

Fig. 28.—On the distal side of the condyle, showing the great extent of the vertical lateral flanges,¹ and the free terminations of the buttresses of the operculum.

Fig. 29.—Through the region of the condyles; the operculum is partially open.

Fig. 30.—On the proximal side of the condyles; showing the way in which the circular marks (*x*) which appear in Fig. 21 are formed.

Fig. 31.—Immediately distal to the basal sclerite.

¹ Owing to the hardness of the chitin of the opercula, I have not succeeded in obtaining completely satisfactory thin sections showing the relations of the labium to adjoining parts.

FIGS. 32—37.—*Euthyris oblecta*, Hincks.—Torres Straits.

Fig. 32.—Part of the frontal surface of a branch, showing two of the large zoëcia (B); *m. c.*, marginal cavity of frond; *calc. p.*, calcareous papillæ supporting the frontal epitheca; *p.*, pores.

Fig. 33.—Basal view of a B-zoëcium from which most of the basal calcareous wall (*b. w.*) has been removed. The tentacles (*t.*), contained in their tentacle sheath, lie in a groove of the compensation-sac (*c. s.*) which bulges out on each side of the tentacles. The operculum (*op.*) is seen partly through the distal wall (*d. w.*).

Fig. 34.—Thick longitudinal section, showing the frontal epitheca (*ep.*) and the basal epitheca (*b. ep.*) held at a distance from the calcareous walls of the zoëcia by the calcareous papillæ (*calc. p.*).

Fig. 35.—Basal view of a B-zoëcium (B) and several others; from the edge of a frond.

Fig. 36.—B-zoëcium and A-zoëcium, calcined. The zoëcia are in contact with their neighbours by small parts only of their walls, which are perforated by communication pores (*c. p.*). The remaining pores (*p.*) are in relation with the cavity beneath the epitheca.

Fig. 37.—Basal view of part of the marginal thickening of an old branch; *z.*, zoëcia; *m.*, free margin of branch; *l., l.*, longitudinal calcareous ridges.

PLATE 17.

FIG. 38.—*Lepralia haddoni*, n. sp.; showing an avicularium (*avic.*) and the two kinds of opercula.—Torres Straits.

FIG. 39.—*L. haddoni*. Basal view of a zoëcium with trifoliate operculum, from the same slide; *m. ov.*, muscles of ovisac.

FIG. 40.—*Smittia reticulata*, J. MacGill.; showing the marginal areolæ (*ar.*).—Naples.

FIG. 41.—*Lepralia pallasiana*, Moll. Young zoëcium. The compensation-sac (*c. s.*) is still small. The pores extend round the distal margin of the operculum.—Naples.

FIG. 42.—*Smittia trispinosa*, Johnst., var. *arborea*, Lev.; showing the compensation-sac (*c. s.*).—Greenland.

FIG. 43.—Diagrammatic transverse section of a Flustrine form, showing the parietal muscles (*p. m.*) inserted into the frontal membrane (*f. m.*).

FIG. 44.—Similar section of a Cribrilina. The frontal membrane is covered by the frontal bars (*f. b.*) or costules.

FIG. 45.—*Lepralia dorsiporosa*, Busk. The distal lobes of the frontal shield (*f. sh. d.*) have nearly united on the distal side of the operculum.—Torres Straits.

FIG. 46.—*Schizoporella sanguinea*, Norm.—Basal view of a zoëcium from which the basal wall has been removed.—Naples.

FIG. 47.—*Schizoporella australis*, Haswell. Operculum with the denticulated condyles.—Torres Straits.

FIG. 48.—*Schizoporella linearis*, Hassall. Showing *avic.*, a gigantic avicularium (= "oëcium," Hincks); *prox.*, proximal end of the zoëcium, into which the polypide extends, beneath the avicularium.—Naples.

FIG. 49.—*Catenaria lafontii*, Aud. Side view, showing the compensation-sac; *z.*, daughter-zoëcia.—Naples.

FIG. 50.—Diagrammatic transverse section of an Umbonuloid Cheilostome. The cavity of the marginal areolæ (*ar.*) communicates with the general body-cavity by the pores *p.*; *f. sh.*, frontal shield, with calcareous tubercles on its outer surface; *ep.*, epitheca; *f. m.*, frontal membrane, or floor of compensation-sac (*c. s.*).

FIG. 51.—Diagrammatic obliquely transverse section through a Microporoid Cheilostome, passing on the right side through the horizontal part of the cryptocyst (*crypt.*), and on the left side through a lateral recess (*l. r.*) containing a depressor muscle (*depr.*) or modified parietal muscle; *f. m.*, frontal membrane (cf. Pl. 18, fig. 58).

FIG. 52.—*Schizoporella linearis*, Hassall. Zoëcium showing the compensation-sac (*c. s.*). The emargination of the frontal shield containing the sinus of the operculum enlarges as it passes through the thickness of the calcareous wall, so that its outline on the inner side of the frontal shield is represented by the line *x.*—Naples.

PLATE 18.

FIG. 53.—*Catenicella alata*, Wyv. Thoms. The zoëcium, which forms the median part of the "globulus," is overlapped by the lateral wings, constituted by *sup. avic.*, the supra-avicularian compartment; *avic.*, the vestigial avicularium; and *inf. avic.*, the infra-avicularian compartment; *x.*, edge of calcareous plate corresponding with *pl.* in Fig. 54.—Victoria.

FIG. 54.—*C. plagiostoma*, Busk, var. *setigera*, MacGill. The avicularia are here of enormous size, and are different on the two sides of the globulus. The roof of the compensation-sac (*c. s.*) is partly a calcareous plate (*pl.*) connected with the calcareous framework of the operculum; the free edge of this plate is seen at *x.* The outer calcareous layer consists of a system of narrow bars separated by large membranous fenestræ (*f.*).—Victoria.

FIG. 55.—*C. hastata*, Busk. The avicularium (*avic.*), although small, possesses a rudimentary polypide (*pol.*). The infra-avicularian compartment (*inf. avic.*) is completely divided into two parts, of which the lower appears to

correspond with the vitta of the next species. The frontal shield (*f. sh.*) is Cribrilina-like.—Victoria (Challenger Collection).

FIG. 56.—*Vittaticella cornuta*, Busk.—Distal end of an old zoëcium with a young "biglobulus;" *j.*, fully formed chitinous joint; *j.*', young joints *h.*, the lateral horns; *h.*', vestigial horn of the proximal zoëcium of the biglobulus; *v.*, vittæ, filled with deeply stained material in the young zoëcia. The compensation-sac (*c. s.*) is fully developed in the proximal zoëcium of the biglobulus, and is quite young in the distal zoëcium.—Victoria.

FIG. 57.—*Micropora*, sp. Basal view, showing the lateral recess (*l. r.*) of the cryptocyst, containing the depressor muscle (*depr.*), and giving off distally a calcareous flange, from which the ocluser muscle (*occl.*) originates.—Torres Straits.

FIG. 58.—*Micropora*, sp. (from the same slide). Frontal view, showing the cryptocyst (*crypt.*) with the two lateral recesses (*l. r.*), and the depressor muscles (*depr.*).

FIG. 59.—*Ichthyaria oculata*, Busk. Zoëcium preceding a bifurcation of the branch, and therefore less curved than most of the zoëcia. The compensation-sac (*c. s.*) opens by the "median" pore (*m. p.*), which is asymmetrical; *s. s.*', sutures in calcareous wall.—S.E. of Buenos Aires (Challenger Collection, Stat. 320).

FIG. 60.—*Calwellia sinclairii*, Busk.—Seen somewhat obliquely. The compensation-sac (*c. s.*) opens by the crescentic median pore (*m. p.*).—S. of Kerguelen Is. (Challenger Collection, Stat. 153).

FIG. 61.—*C. gracilis*, Mapleston. Young zoëcium and parts of its neighbours.—Victoria.

FIG. 62.—*C. gracilis* (from the same slide). Two mature zoëcia, showing the large compensation-sacs (*c. s.*); *c. p.*, communication pores; *prox.*, the narrow proximal part of a distal zoëcium.

FIG. 63.—*Microporella malusii*, Aud. Basal view of a zoëcium which has lost its polypide; *b.*, polypide bud; *c. s.*, compensation-sac, opening by the median pore (*m. p.*); *corn.*, Jullien's "cornicula;" *p. c.*, pore-chambers; *z.*, neighbouring zoëcia.—Naples.

FIGS. 64—66.—*Steganoporella alveolata*, Harmer.—Torres Straits.

FIG. 64.—B-operculum with its ocluser muscles, seen from the inner side, and somewhat distally, so as to be considerably foreshortened; *cond.*, condyles, united to the basal sclerite (*b. s.*) of the operculum by the strong ligaments, *lig.*; *occl.*, proximal ocluser muscle, the tendon (*tend.*) of which is inserted into the ocluser tubercle (*occl. t.*) of the operculum, and gives off a fascia (*f.*) which connects it with the projecting proximal end of the main sclerite (*m. s.*); *occl.*', distal ocluser, inserted into the fascia *f.*'; *x.*, line along which the operculum passes into the roof of the vestibule (cf. Fig. 26).

Fig. 65.—Basal view of a B-zoœcium (basal wall removed). The cryptocyst (*crypt.*) is seen from its basal surface; part of the floor of the lateral recesses (*l. r.*) is broken away. Between the two recesses is the passage (*tube*) by which the tentacle sheath passes to the orifice; *occl.*', distal oclusors, with their transverse fascia (*f.*'); *occl.*, proximal oclusor, with its tendon (*tend.*); *depr.*, depressor muscles of frontal membrane; *div.*, divaricator muscles of operculum; *m. s.*, main sclerite of operculum. The movements of the tentacle sheath are restrained by four delicate muscles, two of which originate from the wall of the tube.

Fig. 66.—Frontal view of a B-zoœcium; *occl. t.*, oclusor tubercle; *lig.*, hinge-ligaments, connecting the condyles (*cond.*) with the ends of the basal sclerite (*b. s.*); *f. m.*, frontal membrane, into which the depressor muscles (*depr.*) are inserted; *m. pr.*, median process, arising from the roof of the tube (cf. Fig. 65); other letters as in Fig. 65.

POSTSCRIPT.—Since this paper was sent to press I have received from Dr. G. M. R. Levinsen a copy of his recently published preliminary note entitled "Studies on Bryozoa" ('Vidensk. Medd. fra den Naturh. Foren. i Kjöbenhavn,' 1902). The paper deals with the morphology to the Cheilostomata, and the questions considered are largely identical with those to which I have here paid attention. Dr. Levinsen's contribution is too important to be discussed within the limits of a postscript.—S. F. H.

July 27th, 1902.

On the Development of Sagitta; with Notes on the Anatomy of the Adult.

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With Plates 19—21.

I. HISTORICAL.

THE development of the Chætogonatha was first investigated by Gegenbaur, but the earliest full account was given by Kowalevsky in 1871 (1). He described the gastrulation, the formation of the archenteric pouches, and the subsequent stages up to the hatching of the young Sagitta. Bütschli studied another species taken off the coast of Norway in 1873 (2), and found considerable difference between this species and that which Kowalevsky had described. The most important new facts which he added were, firstly, the formation of separate anterior cavities of the cœlom, separated off in the earlier stages of the embryo from the archenteric pouches; and secondly, the very early separation of the cells which remain unaltered during the whole embryonic development, and which ultimately give rise to the male and female generative organs.

In the species studied, which unfortunately was not identified, the tissues of the embryo had a much more epithelial

character than those observed by other workers, and the structure of the embryo is therefore almost diagrammatic in its simplicity; the development after hatching was, however not followed.

The most complete and accurate account of the embryology of the group which has yet appeared was published by O. Hertwig in 1880 (3). Besides making a careful investigation of the structure of the adult in several species, he gives a full description of the development, from the first cleavage of the egg up to the tenth day after hatching. He described for the first time the remarkable cleavage, and pointed out that the cells which give rise to the sexual organs are at first two, and later four, and not two or four groups of cells, as was stated by Bütschli. He gives a fuller and more accurate account than his predecessors of the formation of the mouth and alimentary canal, and suggests, without asserting definitely, that the longitudinal septum in the tail region of the adult is formed by a continuation of the alimentary canal into the tail, and not by the prolongation of the splanchnic mesoderm, as Kowalevsky believed.

Hertwig, however, failed to confirm Bütschli's observation on the origin of the anterior pair of coelomic cavities. After the young *Sagitta* has hatched he describes the formation of the body-cavities, which have been obliterated in the embryo, the migration of the genital cells from the splanchnic mesoderm to the body-wall, and mentions that the transverse septa across the body appear also at the same time. He also mentions the formation of a lumen in the intestine, the ciliation of its walls, and refers to the origin of the anus. The origin of the muscles and nervous system is also shortly described, but the account of the development after hatching is altogether much less complete than that of the embryonic stages.

Grassi (4) was the next investigator to take up the study of the Chaetognatha, and his account published in 1883 gives a very full if not always satisfactory description of the anatomy of the adult, but no new observations of importance were

added on the development. Since his time the only important work that has been done on the embryology is a note by Jourdain. This must be referred to, because he contradicts the statements of all previous workers as to the formation of the mesoblast, which he says is not formed by archenteric diverticula, but by a process of delamination between the ectoderm and endoderm. This process is not fully described, and there are no figures, so that his exact meaning is hard to discover.

It will be seen, therefore, that a number of important questions concerning the Chætognatha have been left unsolved; with regard to some points nothing was known, while with others observers have arrived at different results. Leaving aside the note by Jourdain, which reopened the whole question of the origin of the mesoderm and the cœlom, there was nothing certainly known about the mode of development of the transverse septa or of the oviducts and sperm-ducts, both of which are matters of great importance on account of their bearing on the theory that Sagitta is related to the Annelida. It was important also that Bütschli's observation on the formation of head-cavities at an early stage should be, if possible, confirmed, or at least that his account of the development should be reconciled with that given by Hertwig. There are also many minor points; for example, Hertwig in his monograph describes the nervous system as being of two parts, one ectodermal and sensory in function, the other mesodermal and motor. This idea was founded on the anatomy of the adult, but no attempts have been made hitherto to confirm or deny it by study of the embryology.

The present work, undertaken to solve if possible these questions, and to throw light on the systematic position of the Chætognatha, was carried on chiefly at Naples, between October, 1900, and June, 1901, while I occupied the Cambridge table at the Zoological Station. I wish to take this opportunity of acknowledging my indebtedness to Professor Dohrn and all the officers of the station for their unvarying kindness and willingness to give help.

II. MATERIAL AND METHODS.

The material for my work was obtained partly at Naples and partly in the "Pantano" at Faro, near Messina. The Pantano is a lagoon about half a mile in diameter, connected with the Straits of Messina by a shallow canal. In summer, when the water is exceedingly warm, one species of *Sagitta* is very abundant, and during the first two weeks of July, 1900, I obtained a number of eggs in all stages of development by fishing with the tow-net at a depth of fifteen to twenty feet. After allowing the "Auftrieb" to settle for a short time, the bottom layer was drawn off with a syphon and searched with a lens, and in this way quantities of eggs could generally be obtained. I have not been able to determine this species of *Sagitta* with absolute certainty; the adults were generally found at a considerably greater depth than the eggs, although the young occurred at all depths; but as only one species was found, there can be little doubt that the eggs belonged to it. The adult *Sagitta* belonged in all probability to the species *bipunctata*, although it does not entirely agree with Grassi's description; but I have found that this species is very variable, and am inclined to believe that possibly two species have been included under the name "*bipunctata*."

The eggs obtained at Faro developed very rapidly. At 7 a.m. gastrulæ were found, and the young *Sagitta* generally hatched between 6 and 8 p.m. the same evening. The eggs were therefore preserved at intervals during the day in order to get a complete series of the embryonic development. The methods of preservation adopted were (*a*) with sublimate containing 20 per cent. of acetic acid, and (*b*) with osmic acid for a few minutes followed by Müller's solution for several hours. Of these methods, the first gave good results; the second was fairly good for the young after hatching, but the embryos in the shell treated in this way were rarely satisfactory, being very brittle and much contracted. Possibly the osmic acid

remained inside the shell, and therefore its action on the tissues was too much prolonged.

At Naples eggs of several species were obtained, some in May and June, by searching the "Auftrieb" with a lens as at Faro; but since many species are common at Naples, these could not be identified with certainty.

The most satisfactory method was to put a number of Sagitta in a jar with two or three litres of fresh sea water, and next day by drawing off the lower layer of water with a syphon the eggs could be found. In this way a large number of eggs of *S. enflata* were obtained in the autumn of 1900 (from November 1st to December 11th), and again in the spring of 1901. In the cold weather no eggs were laid; the last were obtained on December 11th, and the first in the spring on March 8th. These eggs developed more slowly than those obtained in Sicily, possibly in consequence of the lower temperature, for it was noticed that the development took longer as the weather got cooler. They were also less regular than the Sicilian species. At 9 a.m. all stages from the unsegmented egg up to well-developed gastrulæ were found, and hatching took place usually but not always on the following day. In Sicily it was not found possible to keep alive the young Sagitta more than a few hours after hatching, but the *S. enflata* larvæ at Naples were kept easily for a week, and in one case for fifteen days. They were kept in jars of about three litres, covered with a glass plate, and without any especial care would live for several days. Those that lived for the longest time were kept at an even temperature by placing the jars in running water, and once or twice a day fresh sea water was run in, in such a way as to carry down bubbles of air to aerate the water. The young Sagitta always remained near the surface, so that the water could be drawn off with a syphon from below to make room for the fresh supply. The water was in no case filtered, and the temperature ranged from 16.5° to 18.5°.

I also obtained eggs from *S. bipunctata* at Naples during April and May, and kept the young alive for several days.

Attempts to obtain eggs from *Sagitta minima* and *Spadella draco* were unsuccessful.

At Naples the eggs were generally preserved with concentrated corrosive sublimate, to which a small portion (about 5 per cent.) of acetic acid was added. This method also gave excellent results with the young after hatching; some of these were also preserved with osmic acid, followed by Müller's solution for a day or two. The latter method was more successful with the advanced young when the tissues had already become differentiated; for purely embryonic tissue it gave poor results. Some of the young were also preserved in a solution of potassium bichromate, to which a little acetic acid was added, a method which gave results on the whole similar to those produced by sublimate and acetic acid.

In most cases the material was embedded in celloidin and then in paraffin, and cut in sections $\cdot 004$ or $\cdot 005$ mm. thick; this method facilitated the orientation and gave good results, but to get thinner sections paraffin alone was used. The sublimate material was generally stained in bulk with borax carmine, the rest on the slide with hæmatoxylin. Sections were made of all stages from the young gastrula up to the ninth day after hatching, but the earlier embryonic stages can be most satisfactorily studied in the living egg, which is extraordinarily transparent. After about the sixth hour, however, the structure becomes somewhat complicated, and sections are therefore of great value in confirming and amplifying the observations made on the living embryos and young.

For staining larvæ for mounting whole Mayer's carmalum gave much the best results.

III. EMBRYONIC DEVELOPMENT.

The eggs of *Sagitta* are about 2 mm. in diameter, and are laid in the early morning, and develop floating not far from

the surface. They are enclosed in a firm transparent shell, which may almost fit the egg, as in *S. enflata*, or may leave a large space containing fluid in which the egg is suspended (*S. bipunctata*).

O. Hertwig's account (3) of the early embryonic development, as seen in living embryos, is so complete and accurate that it is unnecessary for me to go over it in detail. I confirmed in all points his account of the cleavage, gastrulation, and formation of the archenteric folds, and further the closing of the blastopore and origin of the mouth, and found that the genital cells appear in the archenteron as he describes. They lie slightly either dorsally or ventrally of the middle line, but I was not able to determine on which side. This eccentricity is shown in one of Hertwig's figures, but not mentioned in his text. In one important point, however, my observations differ from his, and confirm the account of the development given by Bütschli (2). After the mouth has been formed the two lateral lobes of the archenteron become much restricted by the narrowing of the embryo, and their anterior ends are then separated off as distinct cavities by the meeting and fusion of their walls. The two anterior "head-cavities" so produced lie at the sides of the pharynx (Pl. 19, figs. 3-5), and are from the first very small and soon become obliterated; but the mesoblast enclosing them persists, separated off from the remainder, and gives rise to the mesodermal structures of the head. The size of the cavities varies in different species according to whether the egg-shell is large and leaves plenty of room, or the reverse. In *S. bipunctata* they are easily recognisable, while in *S. enflata* they are from the first mere dots, and might easily be overlooked were not other species available for comparison (Pl. 19, fig. 4).

The backward growth of the folds and displacement of the genital cells into the cœlomic cavities takes place as Hertwig describes, and at this stage a faint line can sometimes be seen running back on the dorsal wall of the archenteron from the free end of the folds to the point where the blastopore closed. This looks in the living embryo rather as if the

dorsal wall of the archenteron took some share in the backward growth, but sections lend no support to this assumption. The folds grow back to the posterior end of the archenteron, separating off the coelomic cavities completely, and the folds become pressed together so as to form a thin longitudinal septum running through the animal. As the embryo elongates its cavities become reduced, and this is further increased by the structure of the somatic mesoderm, the inner boundaries of which become irregular, and cracks appear between the cells, so that the layer becomes very indistinct and appears like mesenchyme. This is perhaps the origin of Jourdain's statement that the mesoblast arises by delamination between the ectoderm and endoderm, and not by archenteric diverticula.

The gradual elongation and narrowing of the embryo causes the obliteration of the cavities, and the whole embryo becomes solid, as described by Hertwig. As it grows in length it curls in the shell, and this ventral curvature becomes more pronounced as development proceeds, so that in *S. bipunctata* the tail meets the head (Pl. 19, fig. 6), and in *S. enflata* the embryo is curled through fully a turn and a half before hatching. In the latter species, in which the shell fits the egg, the curvature begins much earlier than in the large-shelled species. When the embryo begins to curl it is easy to get optical transverse sections showing the cephalic mesoderm at the sides of the stomodæum in the head, and in the trunk the two semicircular mesodermal masses separated by the thin endodermal septum, which expands somewhat dorsally and ventrally. My observations on the origin of the ganglionic rudiments and the remaining changes before hatching are so nearly in accord with Hertwig's that it is not necessary to give them in detail. In the species that I studied hatching took place at a time varying from sixteen to fifty hours after the eggs were laid, differing according to the temperature and the species.

IV. FURTHER STUDY OF THE EMBRYONIC DEVELOPMENT BY
MEANS OF SECTIONS.

In the earliest stages of the embryonic development, sections show very little which cannot be seen in the living embryo. The earliest sections made were of the egg shortly after gastrulation had begun, when the archenteric cavity is still small. They show comparatively few large cells, with moderately distinct cell boundaries, and the nuclei arranged close to the outer limits of the cells, in the epiblast, and at the free surface in the cells lining the archenteron. I have not been able to make out with certainty whether the genital cells are already differentiated at this stage, but in one section two nuclei larger than the rest lie side by side close to the archenteric cavity, and it seems probable that these are the genital cells. In sections of a later gastrula there is no very important change; the cells are much more numerous, so that there is an almost continuous band of nuclei round the outside of the embryo and round the archenteron. Two of the latter may be seen to be larger than the rest, and project a little into the cavity. At a stage when the archenteron is divided into three branches by the folds, already four genital nuclei are seen, although the cells still appear as two when seen alive; but as the four are packed so closely together, they would not be easily distinguishable in the living state. (Pl. 19, fig. 7, only one genital cell [*gen. c.*] appears in this section.)

At a period slightly later than this the closing of the blastopore may be seen; it lies now not quite terminally, but slightly in front of the posterior end, but I do not know with certainty whether dorsally or ventrally, owing to the absence of anything to distinguish the dorsal from the ventral surface at this stage. Observations on the living embryo, however, lead me to believe that it is the ventral surface on which the blastopore comes to lie before it closes.

As the embryo elongates, and the different layers come

into such close contact that observations in the living state are more difficult, the sections become more necessary. At a stage when the curvature of the embryo is still not much marked, when the mouth invagination and the head cavities have recently been formed, a transverse section through the head (Pl. 19, fig. 9) shows the epiblast thickest dorsally and laterally, and quite thin on the ventral side. The mouth invagination is seen opening ventrally, and on each side of it are the two masses of cephalic mesoderm in which the cavity is already obliterated, but the nuclei can be seen arranged in a double row, showing how the cavity originally lay between them. These masses of cephalic mesoderm are dorsally in contact with one another, but ventrally they are separated by the mouth invagination, so that their shape in transverse section resembles that of a horseshoe. When traced back into the neck it is seen that they overlap the front ends of the trunk mesoderm, which lies nearer the middle line, so that in some sections parts of both can be seen at once, and hind ends of the head mesoderm masses lying at the outer sides of those of the trunk.

The ectoderm of the head, besides its anterior dorsal thickening which gives rise to the cerebral ganglion, is also thickened at the sides in the mouth region, where it forms a layer, two or three cells deep laterally, while it is only one cell thick on the dorsal surface. This thickening is the rudiment of the hood ("Kappe"), the formation of which will be more fully described later.

The alimentary canal in the head fills up a considerable space; it is roughly oval in transverse section just behind the mouth, and consists of a layer of well-defined cells which in the earlier embryos enclose a distinct space, but this disappears later. The alimentary canal in the head is entirely derived from the ectodermal invagination by which the mouth is formed, and the true endodermal part only begins in the neck region. As the embryonic curvature proceeds, the mouth lies more on the ventral (inner) side, and becomes elongated, so as to form a somewhat slit-like

opening lying longitudinally on the ventral side of the head, and it therefore is visible in a number of consecutive transverse sections. When traced backward to the neck the alimentary canal suddenly becomes laterally compressed, and lies more toward the dorsal surface of the body, marking the point where the ectoderm of the stomodæum meets the true endodermic gut. The latter is from the first very narrow, and appears simply as a sort of partition between the masses of mesoblast lying on each side (Pl. 19, fig. 8). Later it becomes still narrower, and forms a thin lamina, slightly thicker dorsally and ventrally than in the middle, and this condition persists until a considerable time after hatching (Pl. 19, figs. 13, 14, *end. sep.*). Just at the posterior end, however, this extreme lateral compression does not take place, and there, up till near the end of embryonic life, the cellular nature of the septum remains visible (fig. 14, *end. sep.*).

The mesoblast of the trunk and tail region is from the beginning sharply distinguished into splanchnic and somatic layers, which have different origins, the somatic being derived directly from the primary hypoblast, the splanchnic from the outer walls of the folds. In early stages a distinct cœlomic cavity is seen enclosed by the mesoblast; it is triangular in transverse section, and placed so that the splanchnic layer forms the base, and the somatic layer the other two sides of the triangle (fig. 8). In these cœlomic spaces lie the genital cells, two on each side, and in contact with the splanchnic layer; and behind them, at a stage when the folds have not yet finished growing backwards, the two cavities open into one another, and a transverse section shows a single archenteric cavity.

This condition with open cœlomic spaces does not, however, persist very long. As the embryo increases in length it becomes correspondingly narrower, and since it is enclosed in the egg-shell its growth is restricted, and apparently in consequence of this its internal cavities become obliterated. In sections rather later than those described above (Pl. 19, figs.

9—12) it is seen that no coelomic space is present, and that the mesoderm forms two strands running through the embryo and separated from one another by the endodermic septum. These mesodermic strands appear in cross-section roughly circular in outline, and near the circumference the cell boundaries are well marked; but it is seen that the nuclei are no longer at the inner ends of the cells, and that beyond them the space which in early embryos was free and unoccupied is now filled with cell-substance, in which the boundaries between the separate cells are very indistinct. This condition appears to have been brought about, firstly, by the compression of the whole embryo and consequent reduction of its cavities; and secondly, by the sinking of the mesodermic nuclei towards the bases of the cells, that is towards the plane separating the mesoderm from the ectoderm. The cell protoplasm of the centre of the mesodermal strands, that is within the ring of nuclei, is of a much looser and more watery character than that near the circumference, which probably accounts for the lack of distinction between the cells; in embryos in which the cytoplasm is less well preserved it tends to form a mass of strands between which are clear spaces. That this is not due entirely to faulty fixation and consequent maceration is certain from the study of the living embryo, in which an exactly similar appearance is seen at this stage as was described in Section III. The basal part of the somatic mesodermal cells is composed of much firmer protoplasm, and the lines separating the cells can be seen distinctly at this stage, and also when the embryo is more advanced. As development progresses the nuclei become aggregated in a dorsal and ventral mass on each side, while in the lateral areas they disappear or become very scarce. Between the nuclei and the base of the cells the protoplasm is becoming modified, so that it now takes a deep brown stain with osmic acid (figs. 13, 14), and a longitudinal section shows that the cells are becoming elongated in the direction of the animal's length. The study of the larva after hatching shows that these dorsal and ventral groups of cells of the somatic meso-

derm give rise to the longitudinal muscles of the body, and that while the greater part of the cell-substance is transformed into muscle, the nucleus and a little protoplasm remain at the inner end and ultimately form the lining of the cœlom. This will be further described in dealing with the sections of larvæ.

It will be convenient at this point to describe more fully the four genital cells, which have now reached their permanent condition. They lie one behind the other in the cœlomic cavity on each side, and when this is obliterated they become embedded in the mesoderm and lie at the sides of the endodermic septum. The male and female cells appear exactly alike, and are characterised by their extremely large nuclei, each of which is generally oval in shape, is enclosed by a definite membrane, and contains numerous nucleoli. The latter are arranged round the edge of the nucleus, close to the nuclear membrane, and they are generally of a rather elongated oval shape. In the remainder of the nucleus a network of fine threads can generally be made out, and since only the nucleoli take up the stain, in some sections the genital nuclei appear to be made up of a large number of very small cells, each with a stained nucleus. Closer examination with a high power, however, shows that this is not the case; the genital nuclei are exactly like those of the rest of the body, except that they are more than twice as large, and the nucleoli are less crowded together. The cell protoplasm of the genital cells is small in amount, and its limits are hard to see, for when embedded in the mesoderm they become enclosed in a sort of envelope of mesodermal cells, which, however, do not at this stage form a definite epithelial sheath. In some sections this mesodermal envelope is not conspicuous, but in others (Pl. 19, fig. 15) small nuclei can be seen closely appressed to the genital cells, and these differ from the other nuclei of the body in staining evenly throughout, instead of consisting of a mass of nucleoli embedded in a colourless matrix.

The ectoderm of the trunk is characterised chiefly by the

development of the ventral ganglion. In the early stages the ventral ectoderm does not differ from the dorsal, but when the archenteric folds have not yet reached the posterior end of the embryo, the ectoderm of the ventral surface from just behind the head along the greater part of the length of the body undergoes changes somewhat similar to those described above in the somatic mesoderm. The nuclei, which at first lie at the outer edges of the cells, sink in till they reach their bases, and the cells at the same time become larger than on the dorsal surface (fig. 8). There is then a proliferation of nuclei along two bands lying in the ventro-lateral areas, and at the same time the whole ectoderm of the ventral half of the body increases in thickness (figs. 10, 11). In this way two bands of closely packed nuclei appear just beneath the developing muscle-cells of the somatic mesoderm, and when the latter are deeply stained the two look very similar, but are always separated by a clear dividing line. In the dorsal half of the body, and also ventrally in the tail, this thickening and immigration of the nuclei does not take place, and the cells retain longer their original character. As development proceeds the ectodermal cells along the mid-dorsal region become very thin, and constitute a mere membrane separating the mesoderm from the exterior; but traced downwards from the mid-dorsal line the ectoderm becomes steadily thicker, and is thickest along the ventral middle line.

V. DEVELOPMENT OF THE YOUNG SAGITTA.

The young Sagitta at the time of hatching differs very markedly in structure from the adult, so that the term larva might almost be used to describe it, especially as a rather sudden change of structure takes place after a few days, which may be compared with a metamorphosis. It is about 1 mm. in length, and so transparent as to be almost invisible, but can be recognised by the naked eye as a minute shining

body, especially when it swims. The larvæ, as a rule, lie motionless near the surface, but swim in a jerky manner when disturbed, just as does the adult Sagitta. In the young at hatching (Pl. 20, fig. 16) the tail-fin is already present to some extent in all cases, but in *S. enflata* there is already a lateral fin beginning to be formed, continuous with the tail-fin and extending forward as far as the genital cells. The head is rounded and separated from the trunk by a slight neck. The line separating the ectoderm from the underlying mesoderm is sharply defined, and the mesoderm forms a solid cord running through the length of the animal, separated into two halves by the endodermic septum (*end. sep.*), which is expanded in the head into a bulb, like a thermometer. The ectoderm of the anterior half of the body is much thickened ventrally, forming the rudiment of the abdominal ganglion (*gang. v.*); and scattered about the surface, especially in the head region, are tactile organs, like those of the adult, consisting of extremely fine bristles arranged in a fan-like manner transversely to the length of the animal (*t. o.*). At the base of each of these organs there are groups of sensory cells, which become more obvious in the later stages; but one of them, which later is very pronounced, is already noticeable just in front of the tail on each side.

Not much of the internal structure can be seen in the living larvæ, but the four genital cells can generally be observed embedded in the mesoderm in close contact with the endodermal septum, just behind the middle of the animal. They are seen better in specimens stained and mounted in balsam, and these show the rudiments of longitudinal bands of muscle just below the ectoderm.

On the second day the larva has slightly increased in length and the fin has grown larger, but has still a very ragged and irregular appearance. Early on the third day no pronounced change has taken place, the fin is larger and more regular, long fin-rays having appeared at even intervals. The muscle-bands are more developed, and show a transverse

striation as in the muscles of the adult. During the third and fourth days very considerable changes take place, giving rise to a type of structure closely resembling that of the adult animal. Up to this time the body has been solid, containing no cavities whatever, and the first indication of the change is the formation of a cavity in the mesoderm of each side of the body and also in that of the head. At the same time the endodermic septum dividing the two mesodermic cords from one another becomes thickened in its anterior half, namely, as far back as the genital cells, so marking out the rudiment of the alimentary canal (*al.*), though a lumen does not appear in it for several days. In the head, however, a cavity appears in the front end of the alimentary canal, which was formed in the embryo by epiblastic invagination, so that a buccal cavity is now present opening by the mouth to the exterior. The rudiment of the hood ("Kappe" or "prepuce") of the adult has been forming as an ectodermal fold, and at this stage has reached considerable proportions, but since it can only suitably be studied in sections it need not be dealt with here. Under it, however, at this stage four or five small hooks make their appearance, the most posterior of which is the longest, while the front one is hardly visible; as they increase in size new ones begin to grow in front, until the normal number for the species is reached (Pl. 20, figs. 18, 19, *hks.*).

The ectodermic swelling in the front of the head, which was already visible in the embryo, has grown larger and forms the rudiment of the brain (*gang. cb.*), while at the sides of the mouth a rounded body appears on each side, which is seen in section to be the lateral ganglion of Hertwig (the vestibular of Grassi). The eyes (*e.*) also appear at this stage as a pair of minute black specks on the surface of the head, so small that their structure cannot be made out or compared with that of the adult, except that, as in the adult, the black pigment is surrounded by an oval transparent area. The muscles of the head also take their definitive form during the third and fourth days; before that time the cells have had

an embryonic character, but now they develop into muscle-fibres arranged as in the adult, with special reference to the movements of the mouth and the action of the hooks.

Behind the head striking changes are also taking place. The abdominal ganglion is becoming somewhat reduced in size, and at each end of it the ectodermal cells assume a remarkable structure, which is especially pronounced in the neck region, but occurs also at the posterior end of the ganglion, between it and the fin, which now extends considerably further forward than the genital cells. The ectodermal cells in the parts indicated lose their cell-contents almost entirely, and become so vacuolated as to resemble the parenchyma of a plant in appearance; this occurs most markedly at the sides of the body, but to a less extent dorsally and ventrally also (Pl. 20, figs. 18, 19, *pt.*). The cells so modified resemble exactly the curious vesicular tissue found in the trunk of *Spadella draco*, the only difference being that the cells in the *Sagitta* larvæ are much smaller and are found in a much less area. This parenchymatous tissue is most prominent about the fourth and fifth days, but it persists as long as I have been able to keep the young animals alive (fifteen days).

In the tail region the fin has become divided into two parts, a tail-fin (*f. t.*), extending a considerable distance forward along the body, but separated by a gap from the lateral fin of each side. In the gap is the large tactile organ mentioned above, situated on a prominent ectodermal swelling (fig. 18, *t. o.*). There is at this stage no trace whatever of the anterior pair of fins of the adult, so that the young animal closely resembles the genus *Krohnia*, in which they are permanently absent, and in which the tail-fin extends forward as in the young *Sagitta*.

The most important changes which take place during the third and fourth days are those affecting the coelom. The solid condition of the mesoderm described above persists until the third day after hatching, but on that day, unless development is retarded by cold weather, the mesoderm cells

begin to separate again into two layers, a somatic layer next to the body-wall, and a thin splanchnic layer surrounding the endoderm, and in this way the coelomic cavities reappear. At first there are two small cavities in the head, and two large ones extending the whole length of the body, those in the head being separated from those behind by a transverse septum. These may be regarded as the same as those formed early in embryonic life, when the head cavities are separated off from the rest of the coelom. After the two posterior coelomic cavities have reappeared, and extend through the whole length of the body, important changes take place in the region of the genital cells, which result in the division of the coelom into an anterior and posterior part, and so give rise to the condition found in the adult.

Up to this time the genital cells have lain embedded in the mesoderm a little behind the abdominal ganglion, and pressed close to the alimentary canal; they are oval in shape, and their longest direction coincides with the long axis of the body (fig. 17). At about this time, however, they begin to change their position, and gradually come to lie with their long axes placed transversely to the length of the animal. They then move slowly across the coelomic cavity until they reach the body-wall on each side, when they again come to lie end to end with their long axes in the same direction as the length of the body. This process is gradual and takes several hours, and while the large and conspicuous oval nuclei travel across, protoplasmic connections can be seen still attaching them to the wall of the alimentary canal, and, when they approach the body-wall, also to the splanchnic mesoderm (Pl. 20, figs. 20, 21).

While they are traversing the coelom the two of each pair lie side by side close together, but not in contact, and during their progress a transverse septum (*sep. tr.*) is formed between them, so that when they arrive at the outer sides, against the body-wall, a septum is left across the coelom, dividing the body-cavity of each side into an anterior trunk and a posterior tail portion, as in the adult. The way in which

this septum is formed is not absolutely clear, for the small size of the animal and the extreme minuteness of the cells made the actual process difficult to follow with certainty. There are two possible means by which such a septum might arise: first, by the splanchnic mesoderm rising up as a fold, and carrying the genital cells across with it till they reached the body-wall (or, what would amount to the same thing, the genital cells moving across and drawing the splanchnic mesoderm with them in the form of a fold); secondly, the septum might arise by the coalescence of cellular envelopes in which the genital cells are enclosed. Such envelopes were described and figured by Hertwig, but in the species which I have studied they have been exceedingly difficult to make out, and I have only rarely been able to see them. By comparing a number of larvæ, both mounted whole and in sections, there seems to be no doubt that the genital cells are enclosed in a membrane which is separate from them, and which contains here and there a few nuclei (Pl. 21, fig. 32). The nuclei are much less numerous than those represented in Hertwig's figures, but they indicate that the envelope is a cellular structure, which is no doubt derived from the mesoderm in which the genital cells have been embedded since an early embryonic stage. When the genital cells move across the body-cavity their envelopes are elongated transversely to the body of the animal, and between the two cells their respective envelopes lie parallel with one another, almost, if not quite, in contact. If when the migration of the genital cells begins their envelopes remain attached to the splanchnic mesoderm at the point between the genital cells, while elsewhere they become free and move across with the cells which they enclose, when the latter have crossed the cavity and reached the body-wall a two-layered septum will have been produced across the body, with the two genital cells lying on opposite sides of it. After comparing a large number of larvæ, alive and stained, it appears to me that the septum is formed in this way, for I have never seen any indication of a fold of the splanchnic

mesoderm, either in the early or later stages of the migration. The body-cavity is narrow dorso-ventrally, so that the cells appear to be in contact with both upper and lower walls during their passage. In many larvæ the septum appears from the first single, but it can be seen in some that it is two-layered, for the two layers are not in contact (fig. 21). This, however, would also be the case if it were formed by a fold. As the genital cells cross the cavity they retain connections with the splanchnic mesoderm for a time, apart from the septum; the anterior (female) cell generally has a strand of tissue crossing to the wall of the alimentary canal in front of the septum, and separated from it by a space, while the posterior (male) has a similar strand behind the septum (figs. 20, 21). These connections are fainter than the true septum, and appear to contain no nuclei; when the genital cells reach the body-wall and take up their permanent position the connections with the splanchnic mesoderm disappear.

At first there are only very few nuclei in the septum, and one of these generally appears at its outer end, next to the body-wall, and this makes that end of the septum very conspicuous as soon as it is complete, almost suggesting at times that a fold of the somatic mesoderm is forming, although this, as a matter of fact, does not take place. The nuclei of the envelopes seem to get collected between the genital cells, and so appear in the septum, while on the outer sides of the two cells no nuclei appear during the migration.

Taking all the facts together, it seems almost certain that the transverse septum is formed in the way here described; but if it should turn out that it is formed by a mesoblast fold, as suggested by Hertwig, the difference is really of less importance than appears at first. The envelopes of the genital cells are mesoblastic structures, derived from the mesoderm in which the cells have been embedded, so that in either case the septum is formed by a double sheet of splanchnic mesoderm in relation with and in consequence of the migration of the genital cells from one side of the body-cavity to the other. In any case the septum is formed

directly in connection with this migration, and in a distinctly different way from that which gives rise to the anterior septum between the head-cavities and the rest of the *cœlom*. The theoretical bearing of these points will be discussed more fully later.

The completion of the septum between the trunk and tail brings to an end the period of development which may be described as larval, for the animal has now essentially the form and structure of an adult. It still differs from the latter in many important respects,—for example, the ovaries and testes of each side are still each represented by a single cell, and there are no genital ducts. The alimentary canal is without a lumen, except in the mouth region, and the anus has not yet been formed. The abdominal ganglion is also enormously larger than in the adult, relatively to the size of the animal; there are no anterior fins, the parenchymatous tissue in the neck region and behind the ganglion is very conspicuous, and many minor differences still exist. The changes, however, which transform the young of this stage into the adult condition are very gradual, extending over several weeks, if not more, and comparatively few could be observed in the young *Sagitta* raised from the egg.

During the first few days after hatching the alimentary canal increases steadily in thickness, and its nuclei become prominent, but the thicker portion ends abruptly opposite the genital cells, so distinguishing the true gut from the longitudinal tail septum, with which it is continuous. A lumen begins to appear in the alimentary canal shortly after the reappearance of the *cœlom*. As the *cœlomic* cavities grow wider the alimentary canal becomes supported by a dorsal and ventral longitudinal mesentery, and about the seventh day its end can be seen bending down in the mesentery to touch the ectoderm. On the eighth or ninth day the anus is formed at the junction of ectoderm and endoderm; it is not exactly opposite the transverse septum, but a short distance in front of it, and a space is left between the two layers of splanchnic mesoderm, extending from the

posterior end of the gut to the transverse septum. The space is in the ventral half of the body; dorsally the longitudinal mesentery extends continuously from the trunk into the tail. This arrangement is also found in the adult *Sagitta*, but is most pronounced in *S. lyra*, in which the distance between the anus and the transverse septum is considerable. When the anus is formed a swelling can generally be seen in the rectal portion of the gut, just in front of it, and the endoderm cells develop cilia, which are especially active in this region. Small particles can be seen circulating in the distended rectum, so that the young animal probably begins to take food at this stage.

On the eighth day a horseshoe-shaped group of nuclei is seen in stained specimens on the back of the head, rather behind the eyes; the two points of the horseshoe are directed backwards, and the nuclei are arranged in a double line. It is probable that this is the rudiment of the ciliated "olfactory organ" (the "corona ciliata" of Grassi).

The abdominal ganglion begins to be gradually reduced in size relatively to the rest of the body, and its elements become more completely separated, so that the nuclei are packed in a dense mass at each side of the ganglion, while the mid-ventral region appears clear, and is composed exclusively of fibres. This gives the ganglion a markedly bilateral appearance, especially in stained preparations.

In the head the muscles have already assumed the arrangement of the adult by the sixth day, and the cerebral and vestibular (lateral) ganglia are now conspicuous. The hood is arranged just as in the adult, and a few days later the cuticular rods which support the teeth ("Stutzplatten" of Hertwig) make their appearance, running forwards and inwards from the region of the hooks, and ending in front below the cerebral ganglion. No teeth, however, are as yet present.

These changes are all practically complete on the tenth or twelfth day, and on a few occasions when I succeeded in keeping the young alive beyond that time no further altera-

tions took place. The further development, leading to the adult condition, has therefore to be studied in specimens taken in the tow-net, and the consideration of it will be postponed until after the sections of the early young have been discussed.

VI. FURTHER STUDY OF LARVAL DEVELOPMENT BY MEANS OF SECTIONS.

The general course of the development after hatching up to the end of the second week has been described in the last section, and therefore only those points will be dealt with here which are better seen in section than in the living or mounted animal. It will be most convenient to consider first the sections of the head at different stages, and afterwards those of the trunk and tail.

A transverse section through the mouth region of a larva killed a few hours after hatching differs little from that through the embryonic head; it is rather smaller, owing to the lengthening of the body at the expense of its width, and it is also becoming more obvious that the ectoderm at the sides of the head is becoming thickened and consists of more than one layer of cells (*hd.*).

On the second day (fig. 22) little change has taken place; the nuclei on the ventro-lateral parts of the ectoderm are more numerous, and at the sides of the head that layer shows signs of splitting, but otherwise the structure is closely similar to that of the first-day larva. By the third day, however, development has proceeded considerably; the tissues are beginning to take on their permanent form, and in consequence the definite structure of the head becomes marked out.

Beginning with the ectoderm, it is seen in fig. 23 that the hood has now been formed by a splitting off of the two outer cell layers at the sides of the head, but on the dorsal surface they remain continuous with the general ectoderm, and so

appear to constitute a fold on each side (*hd.*). In sections taken more anteriorly these two folds are continuous with one another ventrally, and form a membrane covering the anterior part of the mouth; but further back they do not reach so far down, and appear in section as flaps at the sides of the head (figs. 23—26). There is a tendency for the two layers of the hood to split apart from one another in the region of its insertion, and so give rise to a cavity (*hd. cav.*) which is sometimes large in sections, but in life apparently always narrow if present at all.

It is also seen that the epidermis is thickened under the hood, especially near the insertion of the latter; this condition persists in the adult in the anterior part of the head, and, since the thickening is in just the region from which the hooks ("Greifhaken") grow out, it is possible that it is connected with their formation. Just at the corners of the mouth there is an aggregation of nuclei which will give rise to the lateral ganglia (*gang. l.*) (called vestibular by Grassi). The nuclei plainly belong to the ectoderm, and some sections show the rudiments of the ganglia extending further forward than the mesoderm, and therefore Hertwig's supposition that these ganglia belonged to the mesoderm must be regarded as incorrect. On the dorsal surface a lens-shaped thickening of the ectoderm is now visible on each side, forming the rudiment of the eyes which appear in this stage (*e.*).

The mouth and alimentary canal of the head have not altered much on the third day, but the cell layers are becoming more definite, and a small cavity has already appeared.

The mesoderm is beginning to be transformed chiefly into muscle, but on the third day this change is not yet complete; the cells, however, have a looser and less regular appearance than before.

During the fourth and fifth days the structures which were outlined on the third day are further developed, and by the sixth day they are approaching completion. Figs. 24—26 represent three sections through the head of an individual of

this age. The most prominent objects in the first two of these are the vestibular ganglia lying at the sides of the mouth, which are now almost completely developed, and consist of an inner mass of "Punktsubstanz," surrounded by a layer of deeply staining nuclei.

The cerebral ganglion is also complete by this time; it lies at the anterior extremity of the head and to a large extent in the hood, in which it extends ventrally nearly as far as the mouth. In the adult it becomes more restricted, as does the abdominal ganglion. At this stage it consists of an outer layer of nuclei covering a deeper mass of "Punktsubstanz," but the latter is more restricted than the nuclear layer. Fig. 25 shows on the dorsal surface of the mouth region two large masses of nuclei in the ectoderm, representing the eyes (*e.*), while in this figure and in fig. 24 the hooks are seen lying at the sides of the head, covered by the hood (*hks.*).

The development of the mesodermal structures has now proceeded considerably, and the chief muscles of the head are already differentiated. Their general arrangement is indicated in fig. 26 (*h. mus.*), where it is seen that they do not differ much from those of the adult. Behind the mouth the pharynx lies near the dorsal surface, and when the muscles are formed a cavity appears just below it, which corresponds with the cavity called by Hertwig the head cœlom. A little further forward a cavity is present on each side more dorsally (figs. 24, 25, *b. c. l.*), and these are undoubtedly cœlomic. Already about the third day the pharynx is seen to be surrounded by a layer of cells which belong to the mesoderm, corresponding to the splanchnic layer (fig. 23), but, as in the trunk, these cells later become so closely connected with the alimentary canal as to be indistinguishable from it. About the fifth day, however, a cavity appears on each side between these cells and the outer layer of cephalic mesoderm, which is now being changed into muscles. This cavity is the head cœlom, and that below the pharynx further back appears to be also cœlomic, and to be formed by the coalescence in this region

of the originally paired head cavities. At this stage the head-cœlom is clearly separated from the hood cavity, but later the latter seems to be obliterated, and the cœlom extends into the base of the hood.

The alimentary canal of the head has now a well-defined cavity, which extends back into the neck; in its walls cell boundaries can no longer be distinguished, and they appear as continuous masses of tissue with nuclei at intervals.

The latest sections made were of ninth-day larvæ (Pl. 20, fig. 27); these show the same structures as are described above, but are further advanced, and approach more nearly the adult condition.

Before turning to the structure of the trunk, that of the neck must be shortly described. When a series of sections of the sixth day is followed backward, a little behind that represented in fig. 26, a pair of oval cavities appear suddenly, one on each side, between the pharynx and the epidermis (fig. 28). These are the anterior ends of the trunk cœlom, which diverge a little in front (cf. the figures of the living head), and are overlapped both dorsally and ventrally by the mesodermal structures of the head. Followed back, however, the latter disappear rapidly, and at the same time the pharynx bends towards the ventral surface, so that the two cœlomic cavities, which take up the greater part of the section, are separated dorsally by a thin mesentery. The epidermis begins at this point to assume the vacuolated structure described above, but this is, as a rule, not well shown in sections owing to shrinkage; sometimes, however, as in fig. 29, *p. t.*, it is well seen. A paired mass of nuclei is also seen in the dorsal epidermis, which is the rudiment of the "olfactory organ" ("corona ciliata," *ol.*). A little further back the ectodermal pharynx joins the true gut, the change being marked by the alimentary canal becoming very narrow and lying like a septum from the dorsal to the ventral surface, supported at each end by a short mesentery, instead of being thick-walled and lying against the ventral body-wall.

The trunk and tail as seen in sections now remain to be described. On comparing a section through the trunk of a first-day larva with that of an embryo, the only differences are that the animal is more flattened dorso-ventrally, the cells which will give rise to the ganglion are more marked off from the epidermis, and the cells of the somatic mesoderm are more collected together. Traced backward, the ventral thickening dies out, and the animal becomes a narrower oval in section, with epidermis one cell thick covering the mesoderm dorsally and ventrally; but laterally, where the fin is beginning to form, it remains slightly thickened. A little behind the ganglion the genital cells are seen pressed against the septum which divides the mesodermal strands. Behind them the animal gets gradually thinner, and more compressed dorso-ventrally, but otherwise there is no important difference of structure.

On the second day (Pl. 21, fig. 31) the most noticeable change is the reduction in size of the ventral ganglion. This has taken place by a shrinking of the cells; the nuclei remain as before, aggregated ventro-laterally, but the cell protoplasm which was so conspicuous between them and the epidermis has now largely disappeared. In the mid-ventral region, just below the alimentary canal, the rudiment of the fibrous part ("Punktsubstanz") of the ganglion is appearing. Some of the nuclei (*gang. nuc. 2*) in the ganglion are noticeably larger than the others. In some specimens, both alive and in section, round cavities appear in the ganglion, one or two on each side, but as they are rare and seem to occur irregularly in the few cases where they are present, they are probably pathological. A change has also taken place in the mesoderm; some of the nuclei of the somatic layer have again moved inwards owing to the growth, between them and the outer limit of the mesoderm, of a compact, rather faintly staining mass of tissue, most of which gives rise to the muscles (*mus. c.*). Behind the ganglion region the only point of importance is the further lateral outgrowth of the epidermis into the rudiment of the fin as a sort of lateral fold.

In the third-day larva it is seen at once that from the head as far as the genital cells the septum dividing the two halves of the mesoderm is much thicker in the middle, though dorsally and ventrally it narrows to a thin mesentery. It also contains numerous nuclei instead of the small number that were scattered at intervals up to this time. In the mesoderm the muscles are now well advanced, and appear as three pinnate groups (*mus.*) in each quadrant, especially in the tail region, where they are relatively larger (fig. 32). Over each group lies a nucleus, which is either that of the muscle-cell or belongs to a very slender peritoneal epithelium, but from sections of the adult I am inclined to doubt the existence of the latter.

As the nature of the muscles and coelomic wall is of great importance in fixing the systematic position of the Chaetognatha, it will be well at this point to review the whole evidence. It is seen in figs. 13, 14 (Pl. 19) that the nuclei of the somatic mesoderm, although collected into four groups at the outer sides of the mesodermal strands, do not lie actually in contact with the junction between the mesoderm and ectoderm. The nuclei in each group lie on the inner face of a mass of tissue, which stains more deeply than ordinary cell protoplasm, and fig. 30 (Pl. 21) shows that this tissue is the rudiment of the muscles. It is found, however, that it never contains nuclei, while the nuclei which appear on the surface of each mass seem to belong to the cells which compose it, lying at their inner ends. As the somatic mesoderm is traced through the later stages the same condition is found; in the first and second day after hatching the only difference is that the tissue beneath the nuclei is enlarging and becoming fibrous, while on the third and subsequent days the muscles, with their pinnate fibres, are fully formed.

When a section of an adult Sagitta is examined (cf. fig. 36) the muscles have increased largely in number, but otherwise no alteration is found; the nuclei still appear at the inner ends of the pinnate groups of fibres. In some cases, e. g. in the series from which figs. 36, 37 were drawn, the muscles

are often alternately large and small; the large ones run up into cells with distinct cell boundaries on each side, though continuous with the muscles below, while the smaller ones end abruptly. The conclusion from these facts is that in Sagitta the muscle-cells retain their protoplasmic character, with the nucleus at the inner surface next to the cœlom, and that the muscular part of the cell has a greater length than the protoplasmic portion, so that in transverse sections not all the muscles appear to be continuous with the protoplasmic part. Since the cœlom is bounded by muscle-cells there is no separate peritoneal epithelium, and this conclusion is supported by the way in which, in adult Sagitta, the whole mesoderm passes into an epithelium one cell thick at the lateral lines, and by the fact that in stained larvæ nuclei are seen in rows along the muscles, but not between them.

Returning to the structure of the third-day larva, it is found that the rest of the mesodermal strands is much looser, and a cœlomic cavity is being formed, but is still crossed frequently by strands of protoplasm. In the ganglion no important alteration has occurred, but the nuclei seem to have increased in number, and the fibrous portion is becoming more conspicuous between the two groups of nuclei.

It is during the third day that the genital cells cross to the body-wall and that the transverse septum is formed, but although many series of sections were cut in the hope of throwing light on this question, yet none proved very successful. Fig. 32 represents a section through the male genital cells at the beginning of their movement, and shows how they are connected by protoplasmic strands with the mesoderm of the body-wall. In the lower half of the figure a nucleus (*nuc. g. c.*) is seen close to the genital cell, but outside it; and this is almost certainly the nucleus of one of the investing cells which probably give rise to the septum.

In fig. 33 it is seen that the alimentary canal is now quite thick, and the structure of the ventral ganglion is well shown; the nuclear aggregations are sharply distinguished from the epidermis, and connected ventrally by a bridge of "Punkt-

substanz." It also shows two kinds of nuclei in the ganglion; a few large ones, faintly stained (*gang. nuc. 2*), and numerous smaller ones (*gang. nuc.*) which stain more strongly.

After the third and fourth days the changes are gradual and less important. The coelom becomes completely clear, and it also increases in size, while the body-wall becomes thinner. The alimentary canal gets larger and more rounded, so that the distinction between it and the dorsal and ventral mesenteries becomes more obvious; while in the tail, on the other hand, the longitudinal septum becomes exceedingly thin. The genital cells become tightly pressed against the body-wall, and at the same time appear to be somewhat reduced in size. The fins enlarge, and can be easily seen to consist of a fold of the lateral epidermis enclosing a cuticular supporting plate. In the genital region a mass of nuclei is seen on the fin, which are those of the tactile sense-organ present in that region (*t. o.*). The tactile organ mentioned above, lying between the lateral and tail fins on each side, appears as a mass of nuclei in the body-wall just at the posterior end of the lateral fin; the wall is so thin in this region that it is impossible to determine whether they belong to the ectoderm or mesoderm. The nuclei in these sense-organs, like those of the ganglia, seem to stain more deeply than those of the rest of the body. Pl. 21, figs. 34, 35, illustrate the above description, and are taken from sections of a ninth-day larva; but after the sixth day hardly any change seems to take place in the trunk and tail except the further development of the alimentary canal and the formation of the anus.

VII. POST-LARVAL DEVELOPMENT.

In the last two sections the development has been followed from the time of hatching up to the end of the second week, beyond which period no young were ever kept alive. The young at that stage has the essential structure of the adult,

but nevertheless differs from it in several important particulars. With regard to many of these, however, the assumption of the adult form is very gradual, and takes place rather by a process of differential growth than by any real alteration of structure. Such changes as these are the reduction in relative size of the ganglia, more especially of the ventral ganglion, which in a *Sagitta* a week old is nearly half the length of the animal, and also the formation of the anterior pair of fins, which are absent in the larva. These processes of development are comparatively unimportant, and require no further description, but there are a few points in which the young reared from the egg differ very materially from the adult, and these must be followed further. They concern chiefly the reproductive organs and their ducts, and since these have been used as characters for determining the systematic position of the group it is important that their mode of origin should be accurately known. The oldest young which I was able to rear still retained the four primitive reproductive cells undivided, and none showed the least trace of any reproductive duct either during life or in section, and therefore to follow the development of these organs it was necessary to use young specimens taken in the tow-net, in which *Sagitta* of almost all stages were usually abundant.

The youngest individuals which I obtained differed considerably from those reared from the egg, and were obviously considerably older; they were fully twice as long, the anterior paired fins were present, the ganglion much reduced, and the parenchymatous tissue of the neck and behind the ganglion had disappeared, and in every case the genital cells had already divided to form groups of smaller cells. These groups of cells occupy the positions of the primitive genital cells, i. e. there is a pair of groups on each side, one in front of, and the other behind the transverse septum; in section they appear lens-shaped, and lie closely pressed against the body-wall on the level of the lateral fin. In each group all the cells seem alike; they are moderately large, with pro-

minent nuclei, and at this stage also there is no vestige of genital ducts, either male or female. The period in which this condition lasts seems to vary in different species; in small species, such as *S. minima*, very small specimens are found further advanced; while in the larger ones, e.g. *S. lyra*, I have found individuals more than two centimetres long with the genital rudiments in this condition, and with no trace of ducts visible either alive or in sections.

As the further development of the male organs differs greatly from that of the female, it will be convenient to deal with them separately, and since the male portion of the animal is the first to become mature, that will be described first.

Grassi (4) has described the adult generative organs with great detail and accuracy, and made a study of the spermatogenesis; he points out how when the testis reaches a certain size it gives off into the tail-cœlom groups of "sperm-cumuli," which there complete their development, and mentions the fact that the cœlom of the tail becomes divided by secondary septa, the position of which in the large species differs from that of the smaller. He also describes and figures the structure of the vasa deferentia and vesiculæ seminales of the adult, and it is therefore not necessary here to go over these points. As the animal develops, the group of cells mentioned above, which represents the testis, increases in size and grows backward along the body-wall, and at the same time projects somewhat into the cavity, becoming cylindrical in shape instead of lenticular.

In *S. bipunctata* at this stage the secondary longitudinal septa arise, one on each side, dividing each half of the tail cavity into two compartments; but since they occur only in the middle region, and die out both in front and behind, the cavity remains continuous at each end. The septa are exceedingly slender, and contain here and there faintly staining nuclei; I have not been able to discover their mode of origin. When the testis has increased considerably in size it begins to give off masses of cells which fall into the

body-cavity and there develop further, as described by Grassi, and at about the same time the male genital ducts begin to arise. In their first origin the latter appear as a thickening of the ectoderm in the lateral line in the space between the paired fin and the tail fin, just at the hind end of the former. The thickening increases and soon splits into two well-defined layers, and then the upper layer separates from the lower like a blister, leaving a space between them (Pl. 21, fig. 36, *v. s.*). Traced forward this space is found to narrow into a very fine canal, enclosed in a few well-defined cells and lying between the ectoderm and the mesoderm, but from its continuity with the larger space behind there can be no doubt that the whole is ectodermal in origin. This canal runs forward for a considerable distance, and its walls then join the lining of the cœlom into which the canal opens (fig. 37, *v. d.*). As the animal nears maturity the larger space behind increases in size, and at its front end a longitudinal groove appears in the outer wall, along which the opening to the exterior is formed. The whole space forms the seminal vesicle of the adult, and the chitinous "calotte" is formed only at maturity.

In the female organs the course of development is different. After the stage is reached in which the rudiment of the ovary is a mass of similar cells, this mass grows forward and becomes more cylindrical in shape. A differentiation of the cells then begins to take place, those next to the body-wall becoming a sort of epithelium, while those towards the body-cavity become the primitive ova (fig. 38). The latter have two forms; those in the centre of the ovary become columnar, with elongated nuclei, and give rise to the genital epithelium, the cells of which (or some of them) later enlarge and become ova, as described by Grassi. Between this layer and the inner edge of the ovary, already in quite immature individuals larger rounded cells are found, which will be the first ova to mature. Only parts of two or three of the latter appear in fig. 38 (*o.*) because the section is taken close to the base of the ovary, and they occur chiefly near its free end.

Between the epithelial layer which lies against the body-wall and the layer of columnar germinal cells there appears at this stage, at the base of the ovary, a mass of loose tissue with round nuclei (*od. c.*). A rather later stage shows these cells collecting themselves into a double layer, forming the lining epithelium of the oviduct, which, however, up to this point contains no lumen. The oviduct shortly before maturity has two walls,—an inner of cells with round nuclei which as yet enclose no cavity, and an outer which on the inner side forms the germinal epithelium (*germ. ep.*), while on the outside it consists of cubical cells constituting the limiting epithelium above described (fig. 39). The oviduct thus lies wholly within the ovary, except at the point at which it opens to the exterior, where it becomes continuous with the epidermis. There is, however, never any trace of invagination of the epidermis during the growth of the ovary, but as the latter grows forward the loose tissue above described (*od. c.*) grows with it near its outer border, and when the ova become nearly mature gives rise to the oviduct which runs along the whole length. The development of the oviduct is very rapid and takes place just before maturity, and from the facts described it seems certain that it is developed from the ovary itself, and not by an invagination of the body-wall as was suggested by Hertwig, for no trace of such an invagination has ever been seen.

VIII. SUMMARY AND CONCLUSIONS.

The development of the genus *Sagitta* may be summarised in its most important facts as follows:

The cleavage is complete and equal, giving rise to a spherical blastula of apparently similar cells, the nuclei of which lie at their outer ends, and from this a gastrula arises by invagination. When the gastrula is well formed two cells are separated off from its inner layer at the opposite pole from the blastopore; these soon divide into four, which constitute the primitive genital cells, remaining unaltered until a late stage of de-

velopment, and ultimately giving rise to the ovaries and testes. Soon after their appearance a pair of folds arise at the anterior pole, and, growing backward, divide the archenteric cavity into three divisions, of which the middle one forms the alimentary canal, and the lateral ones the coelomic cavities.

The folds push before them the genital cells for some distance, and the latter ultimately come to rest in the coelomic cavities, two in each side. At about this stage the front ends of the coelomic spaces become separated off as head-cavities, and at the same time the ectoderm of the anterior end is invaginated, and meeting and fusing with the endoderm produces the mouth. The blastopore comes to lie a little in front of the posterior end before it closes, and by the lengthening of the embryo in the shell and the general compression caused thereby, all cavities become obliterated.

The ventral ectoderm of the body and that above the mouth become thickened, and many of the nuclei of the former sink in and become aggregated in two ventro-lateral masses against the mesoderm, forming the origin of the ventral and cerebral ganglia respectively, while at the same time most of the nuclei of the mesoderm form four aggregations, a dorsal and a ventral in each section of the mesoderm, extending from the neck to the tail. The embryo assumes a pronounced ventral curvature in the shell, from which it escapes usually not more than two days after the eggs are laid.

At hatching the larva is not more than 1 mm. in length and is rod-like in shape, tapering somewhat from head to tail. There is already the rudiment of the lateral fins in the tail region, and some tactile organs are present on the epidermis. It is solid and contains no cavities; behind the head there is a single layer of epidermis, which ventrally along half the body is much thickened, and constitutes the rudiment of the ventral ganglion, and behind this it is laterally thickened to form the beginning of the fin-fold. The mesoderm consists of two solid strands from neck to tail, the nuclei of

which are aggregated into dorsal and ventral bands on each side, and in these regions some at least of the cells are already elongated and form the rudiments of the muscles. The genital cells lie in the mesoderm against its inner wall, at a point about halfway between head and tail. The endoderm forms a thin septum stretching from neck to tail, and separating the two halves of the mesoderm. In the head the ectoderm is thickened dorsally and anteriorly to form the brain, and laterally to give rise to the hood, while ventrally it is invaginated and forms the solid rudiment of the mouth and pharynx. The two masses of cephalic mesoderm lie at the sides of the latter, and meet one another dorsally.

As development proceeds the fins increase in size, and the paired ones at the sides become distinct from that of the tail; the ganglia become more sharply defined, the endoderm of the body increases in thickness as far as the genital cells, and the muscle-cells become definite muscles with transverse striation, and appearing pinnate in section. The epidermal cells of the neck and those just behind the ventral ganglion assume a vacuolated and parenchymatous nature. In the head the hood is formed by splitting, the mouth cavity opens, the vestibular ganglia are formed from ectodermal cells at its sides, and the eyes and hooks appear. The greater part of the cephalic mesoderm cells become muscles, and then a cavity, probably truly coelomic, appears between them and the pharynx. At the same time coelomic cavities appear in the trunk, and the genital cells migrate across from the splanchnic to the somatic layer, forming as they go the posterior transverse septum, which is probably produced from the mesodermal envelopes of the genital cells.

The coelomic cavities increase in size and their boundaries become definite, but it is not absolutely certain whether they are enclosed by a definite epithelium, or by the cells, the outer ends of which form the muscle-fibres; the latter view seems, however, more probable, since the nuclei bounding the coelom occur always in direct relation with the pinnate groups of muscle-fibres. The alimentary canal acquires a ciliated

lumen and becomes definitely supported by dorsal and ventral mesenteries, and the anus is formed just in front of the posterior transverse septum.

The remaining changes concern chiefly the reproductive organs; the genital cells divide to form four groups of cells, and these grow into cylindrical masses, and form the young ovaries and testes. The latter give off groups of cells into the tail cavities, and from them the sperm-cells arise, while the male genital ducts and seminal vesicles arise by thickening and splitting of the lateral ectoderm of the tail.

The ovaries grow forward as a cylindrical mass of cells, and as maturity approaches the oviducts appear along the outer edges, but completely enclosed in ovarian cells. They seem, therefore, to be formed actually as a cavity in the ovary, and not by ectodermal invagination as has been supposed.

It now remains to consider what bearing these facts have upon the systematic position of the Chætognatha. There can be no doubt that the group is an exceedingly isolated one, and the fact that all the members of it are closely related to one another, and the absence of any skeleton which could be traced back in palæontology, make it peculiarly difficult to determine its relationships with certainty. The Chætognatha have no close resemblances with any other group, and it is frequently found that when judged by different characters their nearest relationships appear in quite different directions, and in consequence they have been associated by various authorities with most of the phyla of the animal kingdom.

In discussing their position, since their important characteristics offer so little help, it is necessary to descend to minor characters and details, and therefore it is impossible to arrive at an absolute certainty, but it is at least possible to discover in what directions the probability of relationship lies. The Chætognatha have been associated, on various grounds, with many different groups, but it will not be necessary to discuss the merits of all. By the majority of authorities on the subject they have been placed either with the Annelida or with the Nematodes, but by some the threefold division of

their body-cavities has been considered as a reason for associating them with *Balanoglossus*, and possibly with the Echinoderms and *Phoronis*. I will discuss first their resemblances with the Annelida.

The resemblances between the Chætognatha and the Annelida were pointed out by Huxley half a century ago, and have since been emphasised by Hertwig and others. In transverse section there is a considerable likeness between *Sagitta* and an Annelid,—such, for example, as *Polygordius*. In each there is a large body-cavity divided by a longitudinal mesentery, which supports the alimentary canal; in each there are four groups of longitudinal muscles, which appear pinnate in section, and in which the fibres are striped. There are no circular muscles, and the epidermis is simple. If the section be taken through the ventral ganglion, the latter corresponds in position with the nerve-cord of *Polygordius*, and from its greater size in the larva may perhaps be regarded as a longitudinal cord which has become aggregated into a single ganglion. The section of *Polygordius* differs from that of *Sagitta* in the possession of oblique septa, nephridia, and blood-vessels.

Further resemblances with the Annelids are found in the cerebral ganglion connected with the ventral by circum-oesophageal commissures, and in the transverse segmentation. It is supposed by the advocates of the Annelid theory that *Sagitta* is an animal of three somites, separated from one another by two transverse septa, one in the neck and the other near the anus. The number of segments has become greatly reduced, but those that remain are closely similar to the typical annelid somite, except that the alimentary canal is not continued into the last one; since, however, embryology shows that the endoderm is continued to the tail, this objection has not much weight. Another important difference is the absence of nephridia in *Sagitta*, and it has been supposed that they are represented in a modified form by the genital ducts. It has been further argued that the hooks of

Sagitta are similar in structure and formation to Chaetopod setæ, which, however, are absent in Polygordius.

One weak point in the above argument is that it is based almost entirely on the anatomy of the adult, and that no account is taken of embryology. There can be no doubt, however, that the development of Sagitta is very much abbreviated, as is indicated by the fact that after the coelomic and enteric cavities have been formed they are closed by the compression of the whole animal, and only open again several days later. Similarly, the reduction of the endoderm to a simple septum, the formation of the ventral ganglion so greatly out of proportion to its future size, and other considerations all point to the abbreviated development. This, however, does not dispose of the objection that the development of the Chaetognatha and Annelida is of a fundamentally different type. The production of the mesoderm in the one case by archenteric diverticula, in the other from pole-cells, shows a wide distinction, though it is possible that the difference is less fundamental than at first appears. However, apart from the earliest stages, the development of Sagitta differs very widely from that of the typical Annelid. There is never a stage which resembles the Trochophore with its large segmentation cavity, its sense-organ, cilia, and head-kidneys, all of which are conspicuously absent in the young Sagitta. But in addition to the differences in embryology, there is grave reason to believe that many of the apparent similarities between the Chaetognatha and the Annelida are superficial, and do not indicate true affinity. The theory is based on the supposed homology of the three segments of Sagitta with Annelid somites, but this homology is by no means certain. Metameric segmentation has arisen in the animal kingdom in very various ways and independently in different groups, and it is very probable that segmentation apparently similar in character may arise in a variety of ways. In examining the embryological origin of the segments of Sagitta, the first thing that is noticed is that they arise at very different times; the head

cavities and the mesoderm associated with them are formed very early in embryonic life, while the division of the rest of the body does not take place until the animal has, in most important respects, the structure of the adult. The separation between head and body is also much greater than between the body and tail, for while the latter are at first identical in essential structure, and in the adult differ only in the absence of the alimentary canal in the tail, yet the head is from the first widely different from the body, and becomes increasingly so as development proceeds.

A further point of importance is the mode of origin of the two transverse septa, and here again there is no agreement. The anterior is produced by the meeting and fusion of the somatic and splanchnic mesoblast at a time when differentiation of tissues has not begun and the mesoblast is still continuous with the hypoblast; while the posterior appears in close connection with the genital cells, and is not formed by the whole thickness of the mesoderm, but probably only by the cellular envelopes of the genital cells, and certainly by the splanchnic layer exclusively.

The most probable origin of the posterior septum is hinted at by Grassi when he suggests that its object may be to separate the male genital organs from the female, and this view certainly gains support from its formation at the time of the passage of the genital cells across the body-cavity. From its mode and time of origin it seems reasonable to regard the posterior transverse septum as essentially part of the reproductive organs, and not closely connected with the general plan of the anatomy; but if this be the case it is necessary to account for the presence of the anterior septum. The most probable reason for its existence would be the early separation of the anterior part of the mesoderm for the production of the important head muscles, and since at the time of its formation in the embryo the body-cavity is widely open, this can only be done by folding off the front end as a separate space, enclosed in its own mesodermal walls. From the time of its separation the development of the head

mesoderm differs greatly from that of the trunk, and in an animal which develops so rapidly as Sagitta it seems natural that the separation should be early and complete.

If this theory be correct it destroys the chief ground for associating the Chætognatha with the Annelida, for the three segments of the body would not be homologous with one another, and therefore cannot be compared with Annelid metameres. Such a separation seems justified by a closer examination of the other points of resemblance, the chief of which are the similarity of the body-cavity and muscles and the comparison of the reproductive ducts with nephridia. The longitudinal muscles of Sagitta certainly have a close similarity with those of Annelids both in structure and arrangement, but from the arrangement of the nuclei which border the cœlom in connection with the muscles it seems probable that there is in reality no cœlomic epithelium in the Chætognatha, but that the body-cavity is bordered by a single layer of cells, the ends of which next to the cavity remain protoplasmic, while the deeper ends develop into muscle-fibres. Such an arrangement exists in the Nematoda, but it differs greatly from the Annelid plan of a definite cœlomic epithelium overlying the longitudinal muscles. Further work on the histology of the group is required before this point can be finally settled.

The relation of the genital ducts to nephridia can be dealt with more certainly, and this also gives evidence against the Annelid theory. In the first place, there is no trace of genital ducts in the early stages, and even in well-grown young they are quite absent until maturity approaches. In this point they differ greatly from nephridia, which appear at an early stage. In their mode of development they differ no less, for, as was shown above, the male ducts arise as epidermal thickenings, and are formed as splits in the ectoderm for the greater part, if not the whole, of their length, while true nephridia are always chiefly mesodermal in origin.

The development of the oviducts is less certain, but lend

no support to the view that they are modified nephridia, for they are almost certainly formed chiefly if not wholly from the actual cells of the ovary, while the external aperture is, like the vas deferens, ectodermal. Although, therefore, the Annelid theory appears at first sight very probable, yet a further examination of the facts on which it is based shows that it is open to grave doubt, and that the resemblance may be very probably due to convergence of type.

Of the remaining chief views of the affinities of the Chaetognatha, those, namely, which place them with the Nematoda or near the Enteropneusta, the latter is open to the objections which affect the Annelid theory; for if the segments of Sagitta are not homologous, but have arisen in different ways and at different times, no ground remains for a theory of their relationship which depends on the view that the three segments correspond with the three divisions of the body-cavity in Balanoglossus and its allies. The only view, therefore, that remains to be discussed is that of affinity with the Nematodes.

The Chaetognatha have been associated with the Nematoda chiefly in consequence of the very similar arrangement of the muscles in four longitudinal groups, and this comparison would be much strengthened if the muscles were shown to be certainly parts of the cells which line the body-cavity.

The following comparisons are more doubtful, but it may be noticed that the vas deferens of Sagitta bears considerable similarity to the excretory duct of a Nematode, for each is ectodermal and lies in the lateral area between the dorsal and ventral muscles, and in rare cases in Nematodes (e. g. *Lecanocephalus*) the duct is said to open into the body-cavity. The oviduct of Sagitta may be compared with that of a Nematode in being continuous with the ovary and formed from the same rudiment. The development of Nematodes is unfortunately imperfectly known, but it is of interest that the genital organs arise, as in Sagitta, by the very early separation of two large cells, which later multiply and form the sexual glands and their ducts, and in the latter

the innermost cells arrange themselves to form an epithelium.

The differences between the two groups are, however, very wide ; in the first place, the body-cavity of the Nematoda is not certainly cœlomic, and the intestine is not supported by mesenteries, nor are there transverse septa. The nervous systems of the two phyla are also very distinct, and when all the points of resemblance and difference are considered together it is evident that there can be no near relationship between them. The conclusion, therefore, must be that if the nearest connections of the Chætognatha are with the Nematodes, yet the two groups have diverged very widely owing to a difference of habit : the Nematoda were perhaps primitive Cœlomata which have become degenerate through parasitism ; while the Chætognatha, if they branched off from the same original stock, have become fundamentally modified for pelagic existence. In the present state of our knowledge it seems safest to regard the Chætognatha as descended from a primitive cœlomate stock, from which the Annelida have arisen on the one hand ; while, on the other, the Nematoda probably branched off, but lost many of their original characters owing to their parasitic habit.

APPENDIX.

ON THE ANATOMY OF SAGITTA MINIMA.

Grassi, in his general account of the anatomy of the group, states that this species has irregular septa between the alimentary canal and the body-wall, but admits that he has not been able to throw any light on them by sections.

These septa are very conspicuous in the adult *S. minima*, and form the readiest means of identifying it at a glance. They occur especially in the anterior part of the body, but at intervals through the whole trunk region ; they are quite

irregularly arranged, and not at all accurately transverse (Pl. 21, fig. 40, *sep. sp.*). In the places where the alimentary canal is not in contact with the body-wall the septa never cross the space between them; they only touch the body-wall where the gut is in contact with it. In very young minima they are not present, and after studying sections of various ages I have come to the conclusion that they are not septa, properly speaking, at all. The alimentary canal in this species differs from that of others in being very wide, almost entirely obliterating the coelom; but this is less conspicuously the case in the young. If a series of sections of various stages is examined it is seen that the widening of the alimentary canal is due to an enlargement of its walls, while the lumen remains narrow as in other species. The cells composing the walls become exceedingly large, and ultimately lose most of their protoplasm; this change takes place soonest in the lateral region, while dorsally and ventrally the cells remain less modified. At length, when the animal is sexually mature, and its life is probably nearly at an end, the alimentary canal consists of the inner wall surrounding the lumen, and an outer wall, which is largely in contact with the wall of the body; and between these two is a cavity crossed at intervals by sheets of tissue, which are the remains of the cell walls of the endodermic cells. These sheets are irregular in their distribution, and seem to correspond in every way with the "septata" seen in the living animal (figs. 41, 42). At the posterior end of the alimentary canal, between the ovaries, this vacuolation and degeneration does not take place, so by following forward a series of sections of an adult *S. minima* the whole course of changes can be observed. To test this further, some living *S. minima* were put in a watch-glass with sea water in which carmine particles were suspended, and as the animals swallow water at intervals the carmine was taken into the gut and gradually travelled backwards to the rectum, which it reached in periods varying from five minutes upwards, and was finally expelled at the anus. During the whole of its

passage it always kept exactly to the middle line, never passing laterally into the cavity at the sides, and the drop of water containing the carmine seemed to push apart the walls enclosing the lumen of the gut, which was otherwise almost or entirely obliterated by the walls coming into contact.

The "septa" mentioned by Grassi seem, therefore, to be the remains of the endodermal cells, and are not any way to be regarded as mesodermal septa supporting the alimentary canal.

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-

EXPLANATION OF PLATES 19—21,

Illustrating Mr. L. Doncaster's paper "On the Development of Sagitta."

The figures of sections were drawn with the help of a camera lucida, using a $\frac{1}{6}$ -inch or a $\frac{1}{12}$ -inch objective. The remaining figures are mostly drawn from the living animal, and the camera lucida was not used. The magnifications given are approximate.

EXPLANATION OF THE LETTERING OF THE FIGURES.

a. Archenteron. *al*. Alimentary canal. *al. div.* Diverticula of alimentary canal in *S. minima*. *b. c.* 1, 2, 3. Head, trunk, and tail cœlom. *bl*. Blastopore. *c. p.* Cœlom pouch. *e*. Eye. *ect*. Ectoderm. *end. sep.* Endodermic septum. *epi*. Epidermis. *f. p., f. t.* Posterior and tail fin. *f. sk.* Fin skeleton. *f. r.* Fin ray. *f. a.* Archenteric fold. *gang. cb., gang. l., gang. v.* Cerebral, lateral, and ventral ganglia. *gang. nuc.* Nuclei of ganglion. *gang. nuc. 2.* Large nuclei of ganglion. *gang. fib.* Fibrous layer ("Punksubstanz"). *gen. c.* Genital cells. *gen. nuc.* Nucleus of genital cell. *germ. ep.* Germinal epithelium. *h*. Head. *hd.* Hood. *hd. cav.* Hood-cavity. *h. mus.* Head muscles. *hks.* Hooks. *mes. 1, 2.* Mesoderm of head, trunk. *mes. so.* Somatic mesoderm. *mo.* Mouth. *mus.* Muscles. *mus. c.* Muscle cell. *mus. nuc.* Nucleus of muscle cell. *nuc. g. e.* Nucleus of genital envelope. *o.* Ovum. *o. d. c.* Lining cells of oviduct. *ol.* Olfactory organs. *ov.* Ovary. *ph.* Pharynx. *p. t.* Parenchymatous tissue. *sep. l.* Longitudinal septum. *sep. tr.* Transverse septum. *sep. sp.* spurious septum (in *S. minima*). *sh.* Egg-shell. *t. o.* Tactile organ. *v. d.* Vas deferens. *v. s.* Seminal vesicle.

PLATE 19.

FIG. 1.—*S. bipunctata*. Gastrula with genital cells. $\times 220$.

FIG. 2.—*S. bipunctata*. Formation of archenteric folds. $\times 220$.

FIG. 3.—*S. bipunctata*. Embryo with head-cavities recently formed. $\times 250$.

FIG. 4.—*S. enflata*. Formation of head-cavities.

FIG. 5.—*Sagitta*, sp. Optical transverse section of embryo through recently formed head cavities. $\times 250$.

FIG. 6.—*S. bipunctata*. Embryo shortly before hatching. $\times 220$.

FIG. 7.—*S. bipunctata*. Longitudinal section of embryo during formation of the archenteric folds. $\times 375$.

FIG. 8.—*S. bipunctata*. Embryo at stage of Fig. 3. Transverse section through the middle of the body. $\times 500$.

FIGS. 9—12.—Four transverse sections through an embryo at about the stage of Fig. 3. Fixed with osmic acid. $\times 600$.

Fig. 9.—Through the head.

Fig. 10.—Through the ventral ganglion.

Fig. 11.—Through the genital cells.

Fig. 12.—Through the tail region. The archenteric folds have not yet grown back to this level.

FIG. 13.—*S. bipunctata*. Longitudinal horizontal section through a late curled embryo, cutting the head and trunk. $\times 375$.

FIG. 14.—Section through the same embryo, showing the genital cells below and the tail above. The embryo is cut twice owing to curvature. $\times 375$.

FIG. 14 *a*.—Diagram to show the planes of section of Figs. 13 and 14.

FIG. 15.—*S. bipunctata*. Section of embryo at about the same stage as Figs. 13, 14, showing the genital cells and their envelopes. $\times 900$.

PLATE 20.

FIG. 16.—*S. bipunctata*. Larva just after hatching. $\times 200$.

FIG. 17.—*S. enflata*. First day. Genital cells. $\times 500$.

FIG. 18.—*S. enflata*. Fourth day. Ventral view. The eyes are seen through the head. $\times 200$.

FIG. 19.—*S. enflata*. Fourth day. Head and neck, lateral view showing parenchyma. $\times 600$.

FIG. 20.—*S. enflata*. Third day. Genital region in stained specimen, showing envelopes of genital cells. $\times 800$.

FIG. 21.—*S. enflata*. Fourth day (a late specimen). Showing migration of genital cells. $\times 600$.

FIG. 22.—*S. enflata*. Second day. Transverse section of head. $\times 1000$.

FIG. 23.—*S. bipunctata*. Third day. Transverse section of head. Only half of the section is represented. $\times 900$.

FIGS. 24—26.—*S. enflata*. Three sections through the head of a sixth-day larva. $\times 375$.

FIG. 24.—Through the mouth.

FIG. 25.—Through the eyes and beginning of the pharynx.

FIG. 26.—Through the crescent-shaped transverse muscle in the posterior part of the head.

FIGS. 27—29.—Three sections through the head and neck of a ninth-day *enflata* larva. $\times 375$.

FIG. 27.—Through the posterior region of the head.

FIG. 28.—Through the beginning of the neck.

FIG. 29.—Through the neck.

PLATE 21.

FIG. 30.—Part of longitudinal horizontal section through embryo shortly before hatching, to show development of muscles. $\times 1000$.

FIG. 31.—*S. enflata*. Second day. Transverse section through region of ventral ganglion. $\times 750$.

FIG. 32.—*S. enflata*. Third day. Transverse section through genital cells. $\times 900$.

FIG. 33.—*S. enflata*. Fourth day. Transverse section through ventral ganglion. $\times 750$.

FIG. 34.—*S. enflata*. Ninth day (same specimen as Figs. 27—29). Transverse section through ventral ganglion. $\times 375$.

FIG. 35.—Same larva; section through female genital cell. $\times 375$.

FIG. 36.—Transverse section through seminal vesicle of a young *S. enflata*. $\times 550$.

FIG. 37.—Vas deferens of same specimen. $\times 550$.

FIG. 38.—Transverse section of young ovary in same specimen. $\times 200$.

FIG. 39.—Transverse section of ovary and developing oviduct in *S. minima* approaching maturity. $\times 550$.

FIG. 40.—Anterior part of adult *S. minima* to show spurious septa.

FIG. 41.—Transverse section of adult *S. minima* near posterior end of trunk. $\times 200$.

FIG. 42.—Section of same near middle of trunk region. $\times 200$.

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On a Cestode from Cestracion.

By

William A. Haswell, M.A., D.Sc., F.R.S.,

Professor of Biology, University of Sydney.

With Plates 22—24.

General Features.

THE Cestode, the results of a study of which are embodied in the present paper, occurs, usually in abundance, in the large intestine of the Port Jackson shark. It is one of these remarkable forms to which attention appears to have been first specially directed by P. J. van Beneden (1 and 2), in which the proglottides are set free from the posterior end of the strobila long before full maturity has been reached, and only attain a stage corresponding to that of the "ripe" proglottides of a *Tænia* after having pursued an independent existence for some considerable time.

The strobila is actively locomotive, and appears to use the suckers more in connection with progression than as organs of permanent attachment. It is only 9 or 10 cm. long in the preserved condition. There is an elongated neck-region with a breadth, in the preserved specimens, of half a millimètre. The four sessile bothridia (fig. 1) are somewhat spoon-shaped, the anterior end being the narrower. The margin of the bothridium is very prominent, finely crenulate, and in the living condition extremely extensile, so that the shape is undergoing constant modification. In preserved specimens

they are about 1 mm. in length. Each bothridium is so directed that a line running along the floor of its cavity in the direction of the long axis, and prolonged forwards, would meet the median axis of the neck at an angle of about 45° . The cavity is not divided or reticulated. At its anterior narrower end, where its margin is lowest, each bothridium bears a small circular accessory sucker.

The last segment (fig. 2) is 5 mm. long and 2 mm. in breadth in the preserved specimens; relatively narrower in the extended living condition.

Separated segments are to be found in abundance along with the entire strobilæ, moving actively through the intestinal contents. In the course of these movements the shape undergoes constant alteration, the phases through which it passes being comparable to those of a Ligula in its most active condition. The anterior end becomes thrust sharply forwards until the "head" becomes long and narrow and pointed, and the "neck" constriction becomes more or less completely obliterated. Then suddenly the anterior end becomes drawn together and thickened to form a distinct rounded knob, constricted off from the rest (fig. 3). The part behind this "head" now becomes drawn forwards, the region immediately following on the head gradually becoming thickened, while the head itself becomes gradually retracted until it nearly completely disappears, to become again thrust forwards as before. The effect of these movements is clear enough. By the thrusting forwards of the narrowed head end, the thick matter contained in the intestine is readily penetrated, the subsequently formed knob at the anterior end then forming a point d'appui, towards which the rest of the proglottis becomes drawn forwards.

These independent proglottides attain a relatively considerable size, the largest being about 11 mm. in length and 1.75 mm. in greatest breadth.

Attention has been recently directed by Lühe (13) to isolated proglottides from *Acanthias*, in which there is a distinct mobile "head" similar to that above described, but

covered with spinules; and a similar case had previously been observed by Pintner.

The early separation of the proglottides in this and other species is obviously correlated with the free locomotive habits of the strobila. With a much longer train of connected proglottides, the posterior loaded with eggs, such movements would be rendered difficult or impossible. The spiral valve in the intestine of the Elasmobranch renders it possible for the separated proglottides, without definite organs of adhesion, yet with an adaptation for creeping movement, to remain within their host until such time as the uterus has become fully charged with eggs.

This Cestode is to be referred to the genus *Phyllobothrium* of P. J. van Beneden. In the definition given by that author¹ the bothridia are described as notched externally, but the notch is not present in one of van Beneden's own species (*P. auricula*), and cannot be looked upon as of generic importance. I propose the name of *P. vagans* for the Cestracion parasite, which appears to be distinct from all the species described hitherto.²

The only species of *Phyllobothrium*, of the structure of which a detailed account has been published, are *P. thridax* and *P. Dohrnii*. These have both been pretty fully described by Zschokke (20, p. 327 et seq.); but, as mature segments were not met with by that author, many features of importance, more particularly in the reproductive apparatus, were overlooked.

Integument and Nervous System.

The cuticle (fig. 4, *cu.*) is homogeneous and not divided into layers. Immediately beneath it are the usual external longitudinal (*e. l. m.*) and circular (*e. c. on.*) layers of muscular fibres. The subcuticular cellular layer is much better developed in the strobila than in the free proglottides, in

¹ 1, p. 120, and 2, p. 123.

² I have not seen the original description of *P. gracile*, Wedl., from *Torpedo marmorata*, but only the brief definition given by Lönnberg (11).

which it has undergone a reduction in thickness. A similar reduction is observable in the internal longitudinal layer of muscular fibres (*i. l. m.*), which are well developed in all parts of the strobila, and very conspicuous in transverse sections owing to their highly refracting character, whereas in the free proglottides they are barely discernible in transverse sections, and in longitudinal appear as a few inconspicuous, often degenerate, fibres.

The nervous system (fig. 3, fig. 4, *n. c.*) is in no way remarkable, consisting of the usual head-ganglion in the scolex, and the pair of longitudinal nerve-cords with their branches and commissures. In the separate proglottides, owing to the reduction in the thickness of the subcuticular cellular and internal longitudinal muscular layers, the nerve-cords come to be situated more superficially than in the strobila. They meet anteriorly in the "head," where there is a slight thickening of the nature of a rudimentary ganglion.

As in many other forms, two of the four longitudinal excretory vessels of the anterior region—the dorsal pair—become reduced greatly in diameter in the posterior proglottides. In the last proglottis these open on the exterior at the posterior end. In the free proglottides (fig. 3) only the ventral pair remain. These are very narrow towards the anterior end, while posteriorly they are very wide and very sinuous; their external openings are situated near together at the posterior extremity. The excretory vessels in general have a wall consisting of a thin layer of fibrillated protoplasmic material; but in the scolex and neck region the four main vessels have a fairly thick layer of longitudinal muscular fibres.

Reproductive Organs.

The reproductive system will be best described first as it appears in its fully developed condition in the free proglottides.

The testis (fig. 3, *te.*) consists of numerous rounded lobes extending from the neck to behind the genital aperture.

They lie in the central or medullary region, and are thus situated on a deeper plane than the vitelline glands. They average about .06 mm. in diameter. Each lobe has a fine, thin-walled efferent duct; the ducts of neighbouring lobes anastomose to form a network. From this network are derived larger trunks, which towards the anterior end, and near the ventral surface of the proglottis, combine together to form a single median vas deferens (*s.d.*). The latter is a closely coiled, widish, thin-walled tube, situated in the middle of the region in front of the genital aperture. Its wall consists of a reticulated material with superficially placed nuclei. No muscular layer was definitely made out, but muscular fibres must be present, as in the living condition the tube is observed to undergo peristaltic contractions. The "prostate" cells described by various authors (see Braun, 5) as occurring in certain Cestodes, are not present. This main testicular duct is always packed full of sperms, and it plays the part of a vesicula seminalis as well as a vas deferens. It terminates by passing through the wall of the cirrus sac and becoming the ejaculatory duct. The cirrus sac has a wall composed of two layers of muscle. Within it, when the cirrus is not protruded, lies coiled up a long tube, continuous internally with the vas deferens. This tube (fig. 5) has a muscular wall, consisting of an outer thicker layer of longitudinal fibres and an inner of circular fibres. Internal to this is a homogeneous cuticular layer, beset on its inner surface in the outer part of the tube with numerous excessively minute spinules. Outside the muscular layer is a layer of cells similar to the myoblasts of the oviduct and vagina. In the space between the wall of the cirrus sac and the enclosed tube are to be observed numerous muscular fibres which appear to run about in every direction.

The outer end of the tube is continuous with the outer extremity of the cirrus sac, and might be described as invaginated within it were it not for the circumstance that its inner end is not free, but passes through the wall of the sac to become continuous with the vas deferens.

The mode of protrusion of the cirrus is rendered evident on an examination of living animals and of sections of specimens with the organs in various states. The strong muscular wall of the cirrus sac contracts, and the narrow outer end with which the invaginated tube is continuous becomes thrust out through the genital opening. Further pressure causes the tube to become evaginated as a narrow cylindrical process, the cirrus, with a double wall, the space between the two walls being continuous with the cavity of the cirrus sac. The retraction takes place through the agency of the muscular fibres that have been above referred to as situated in the cavity of the cirrus sac; when the cirrus is protruded these are put upon the stretch, and each of them is found to be connected internally with one of the myoblasts in the wall of the tube, and to run inwards towards the inner part of the wall of the cirrus sac.

The ovary (figs. 3 and 6, *ov.*), as in many other Cestodes, consists of two large lateral portions and a small median isthmus connecting them together, the whole, on a dorsal or ventral view, resembling a letter **H**, with the limbs thick and near together and the transverse part very short. A transverse section shows that each lateral portion is itself double, consisting of a dorsal and a ventral lamina which coalesce internally towards the isthmus. The margins of the laminae are divided irregularly into a number of rounded lobes, but these divisions are quite superficial, the substance of the lamina consisting of a mass of ova with no trace of a tubular structure, except that irregular fenestræ occur here and there. The ova are somewhat smaller peripherally, largest in the neighbourhood of the isthmus. The mature ova are .01 mm. in diameter; their nuclei, .004 mm.; and their nucleoli, .002 mm. Their cytoplasm appears homogeneous under the highest powers, binding them together in a small quantity of retiform connective tissue. Enclosing the whole ovary is a membrane having the appearance of a condensation of the parenchyma, but perhaps of muscular character.

The isthmus, or connecting part, differs widely from the

rest, and is to be looked upon rather as the beginning of the efferent duct than as part of the ovary proper. It is enclosed in a membrane continuous with that which encloses the lateral portions. The contained ova, instead of being closely aggregated together, are loosely distributed singly or in groups (figs. 11 and 12).

The oviduct begins in a well-developed "swallowing apparatus" (figs. 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, *sw.*), such as has been described in various other Cestodes. This lies on the ventral side of the isthmus of the ovary and opens into its cavity. It is a bell-shaped structure, the wide mouth of which, directed towards the dorsal surface, opens into the cavity of the isthmus of the ovary, while at the opposite extremity a very much smaller aperture leads into the oviduct proper. During life this swallowing apparatus was observed to perform rhythmical pulsating movements, the effect of which must manifestly be to seize the loose ova of the isthmus, one by one, and to pass them backwards along the oviduct. In sections it is found that the wall of the swallowing apparatus is continuous with the investment of the ovary and with the muscular layer of the wall of the oviduct. It has the character of a dense layer of fibres (figs. 13 to 16, *sw. m.*), which, though of extreme fineness, must be muscle-fibres. These are for the most part arranged circularly around the wall of the organ, but some are radial. Surrounding this fibrous layer is a single layer of cells (figs. 13 and 14, *sw. my.*) of irregular shape. Processes pass from these into the fibrous layer, and there can be little doubt that the majority of these cells are the myoblasts of the fibres of the swallowing apparatus. A small number (fig. 13) which give off processes both externally and internally are probably nerve-cells.

Through the oviducal opening of the swallowing apparatus projects for a short distance a sort of plug perforated by a circular aperture. The substance of this plug is continuous with the epithelium of the oviduct; but, though it contains several nuclei (fig. 16), it does not consist, so far as I have

been able to ascertain, of definite cells. On its inner surface, i. e. the surface turned towards the ovary, it is fimbriated (fig. 14), and it is doubtless through the agency of these fimbriæ that the ova are seized during the movements of the apparatus.

The oviduct (figs. 6 and 7, *od.*) runs, at first, nearly straight back from the swallowing apparatus, on the ventral side of the shell-gland and receptaculum seminis, and is joined by the narrow fertilising duct (*f. d.*) from the latter. In this part of its course (fig. 8) it has an epithelium composed of short prismatic cells. Internal to this is a thin cuticle beset on its inner face with numerous slender hairs, resembling cilia in appearance, but non-vibratile, which lie with their apices directed backwards, i. e. away from the ovary, their arrangement thus being such as to prevent the ova received from the swallowing apparatus from passing forwards again towards the ovary. External to the epithelium is a muscular layer composed of external longitudinal and internal circular fibres. Surrounding this is a layer of cells of the same general character as those that surround the muscular layer of the swallowing apparatus. These appear to correspond to the cells which Zschokke (20) looks upon as glandular, and to those which Pintner¹ regards as the formative cells of the swallowing apparatus. In view of Blochmann's² results on the subcuticular muscle, however, and Sabussow's (17) extension of the same view to the reproductive ducts, I am more disposed to look upon these also as myoblasts.

A little behind its point of junction with the fertilising duct the oviduct bends sharply round towards the dorsal side, and is joined by the main vitelline duct at the posterior limit of the shell-gland. From this point it runs forwards for some distance with a sinuous course on the dorsal side of the isthmus of the ovary and of the vagina, and then runs

¹ See Braun, 5.

² F. Blochmann, "Ueber freie Nervenendungen und Sinneszellen bei Bandwürmern," 'Biol. Centralbl.,' xv, 1895.

straight forwards as a cylindrical tube with irregular dilations. As this part of the oviduct contains fully formed eggs, and is something more than a mere passage, it will be convenient to designate it ootype, or primary uterus. Anteriorly it opens into the secondary uterus by a longitudinal slit, the extent and position of which vary in different specimens, situated on one side of the vagina.

After it becomes joined by the main vitelline duct, the oviduct changes its structure, the cuticular hairs are lost, and there is no epithelium, the wall of the duct now consisting of cuticle, muscular layer, and layer of myoblasts.

The uterus (figs. 3, 6, and 18, *s. u.*) is a cylindrical undivided chamber, extending from the level of the reproductive aperture to the interspace between the anterior portions of the lateral wings of the ovary. It has a lining membrane composed of a single layer of cells. It has no natural external aperture, but dehisces by the formation of a longitudinal slit along nearly the whole, or only a limited part of the length of its ventral surface. This dehiscence readily takes place when the specimen is manipulated, more especially when it is placed in sea-water, when the eggs are observed to be suddenly discharged with the appearance of a white cloud.¹

The shell-gland is a compact oval body, .18 mm. in length, which surrounds the oviduct where the vitelline duct joins it.

¹ Shipley, in his description of the worms collected by Dr. Willey (19), in referring to a species of *Phyllobothrium*, states that in the oldest proglottides the uterus had ruptured "about the centre of the dorsal surface." But there can be no doubt that the surface on which the dehiscence takes place is the ventral, and not the dorsal. This is made perfectly clear in the case of the *Cestracion* species by the relative positions of the various parts of the reproductive apparatus—as, for example, the vagina and vas deferens—and by the disposition of the longitudinal vessels of the excretory system. It may be remarked, however, that in the Australian land Planarian (*Geoplana Mortoni*) Steel has confirmed by observation on the living animal Dendy's description of the rending of the dorsal body-wall on the discharge of the egg-apsoles ('Proc. Linn. Soc. N.S.W.,' 1900, p. 573, pl. 34, fig. 10, and pl. 41, fig. 6).

Its cells, several hundred in number, are arranged in a radiating manner round the oviduct, their narrow inner extremities evidently acting as ducts by which the secretion is discharged. Their nuclei are large, a little less than $\cdot 005$ mm. in diameter. Between the cells are a number of smaller nuclei indicating the presence of a certain amount of inter-cellular tissue.

The vitelline glands (fig. 3, *v.*) extend throughout a narrow belt of the lateral regions of the body from the neck to the posterior end. The lobes are spherical or subspherical in shape, and average about $\cdot 03$ mm. in diameter. Each lobe has its slender duct, which joins those of neighbouring lobes to form larger ducts, and these again combine to form the main lateral ducts (fig. 7, *v. d.*). These converge from both sides towards the middle line, running on the ventral side of the ovary, and finally unite to give rise to an impaired main duct, situated slightly to the right of the middle line. This runs backwards and joins the oviduct as already described. Near its termination it is usually distended with yolk, and this dilated part (figs. 7, 8, and 9, *v. r.*) ($\cdot 03$ mm. in diameter) might be looked upon as a yolk-receptacle. It is followed by a constricted part with thickened walls (fig. 8, *v. r. c.*) through which the yolk cells can only pass singly to enter the oviduct. The yolk matter leaves the lobes of the glands in the form of very regular spherical masses $\cdot 012$ mm. in diameter, each of which contains one, or sometimes two, rounded bodies which, as they are capable of being stained, though only slightly, are very liable to be mistaken for nuclei. These bodies will be further referred to in the description of the egg. Meanwhile it is of importance to emphasise the fact that they are not nuclei, and that the vitelline masses in which they are lodged are not cells.¹

The wall of the vitelline ducts consists of fibrillated protoplasmic material with nuclei at intervals. In the main duct

¹ This is contrary to what is usually stated of Cestodes in general. Braun, for example, states: "Die Ansicht Moniez's dass die Dotterzellen keine echten sondern nur Scheinzellen seien entbehrt jeder Begründung (5, p. 1468).

the wall is thicker, and contains a large number of superficially situated nuclei.

The vagina (figs. 6, 9, and 10, *va.*) opens into the shallow genital cloaca by a narrow aperture immediately in front of the male aperture. The terminal part is somewhat dilated. From this point it bends round the sac of the penis as a narrow tube, which dilates again to a diameter of about .05 mm., as it runs straight backwards immediately above (i. e. on the dorsal side of) the secondary uterus. When it reaches the region of the ovary it again becomes narrower and more sinuous. Eventually passing backwards on the dorsal side of the isthmus, it becomes somewhat dilated again to form a vesicle, the receptaculum seminis (figs. 6, 7, 9, 10, 11, and 12, *r. s.*). From the rounded posterior end of this a narrow duct, the fertilising duct (*f. d.*), runs to join the oviduct.

In the posterior part of its extent the vagina has a thickish muscular wall consisting of external longitudinal and internal circular layers. Internal to this is a cuticle beset with exceedingly minute spinules. External to the muscle is a layer of cells resembling those cells of the oviduct which I have supposed to be myoblasts. Anteriorly the muscular layers become reduced, and longitudinal fibres alone are present. The fertilising duct resembles the oviduct in structure, but the cuticular hairs are absent. In the posterior proglottides of the strobila (fig. 2) all parts of the reproductive apparatus are represented, though neither the male nor the female organs are mature, and there are no eggs in the uterus. The latter has a comparatively narrow lumen surrounded by a thick layer of small cells; its aperture of communication with the primary uterus is already developed. In more anteriorly situated proglottides the uterus is represented by a solid cord of small cells running along on the ventral side of the vagina.

Development.

In the case of *P. Dohrnii*, Zschokke (20) states that the formation of eggs begins in the posterior proglottides of the strobila. In the form now under consideration this is not the case, eggs only occurring in well-developed free proglottides.

The only recorded observations on the development of any member of the genus appear to be a few notes on *P. thridax* by Moniez (14, p. 28). I can trace no correspondence whatever between the statements there made and what I have been able to observe in the species from Cestracion.

The primary uterus contains only eggs with unsegmented ova. The entire egg is in the form of a thick spindle about .045 mm. in length and .021 mm. in greatest breadth. The shell is at this stage not yet fully solidified, so that the shape is readily modified by pressure, and the eggs tend to adhere together in masses. The shell consists of two distinct layers—an outer homogeneous and an inner made up of fine fibrillæ—which run in the direction of the long axis of the egg.

The completed egg in the primary uterus contains (1) the unsegmented ovum; (2) a large number of small, bright globules (3) one, or, more commonly, two, larger rounded masses. The last two are the substance of the vitelline spherule. When the eggs are acted upon by any weak acid the small globules tend to run together into larger (2 [14], p. 28) masses, and eventually these pass out through the shell at the ends of the egg, so that in preparations fixed and stained by any of the ordinary methods this constituent of the egg becomes completely lost, there being left behind merely some irregular granular matter, in which, presumably, the globules were enveloped. These globules, from their appearance and behaviour, are most probably composed of oily matter.

The larger bodies derived from the yolk (see fig. 20) are of an entirely different character. They are solid masses having the central hilum and concentric lamination characteristic of the calcareous corpuscles. They become coloured, though not strongly, by staining agents, the central mass colouring

first. In fixed and stained preparations they become much altered, having apparently become partly dissolved, and the concentric lamination being no longer discernible, might very easily be taken for nuclei. Like the oil globules, these bodies consist, doubtless, of food materials; but both these ingredients of the yolk persist, not greatly diminished in bulk, to the most advanced stage observed. Nothing was made out with certainty as to the processes of maturation and impregnation. The oosperm does not differ to any appreciable extent from the ovarian ovum.

Very few, if any, unsegmented ova were found in the secondary uterus. No definite history of the process of segmentation could be traced, as there seemed to be great variation in the details. The first two segments (figs. 20 and 21) are equal. One of these, or both, become divided into two equal parts (figs. 22 and 23), and from the three or four equal, or nearly equal cells thus formed, a number of smaller cells become segmented off (figs. 24, 25, and 26). Eventually the larger cells become reduced by division until a blastoderm is formed consisting of a disc of small cells (figs. 27, 28, and 29), which are very irregular in size and shape, and present no definite arrangement. This disc becomes thickened to form a rounded mass, on the surface of which appears here and there a flattened cell. In this stage there appears to be no further cell-differentiation, except that there are present, in the most advanced embryos, one or two pairs of very small cells that become more intensely stained than the rest. It is conjectured, from their arrangement in pairs, that these are the cells destined to develop the hooks.

No hooked embryos were found in the uterus of any of the numerous specimens examined. But of a number of eggs which had been kept in pure sea-water for five days, a large proportion (figs. 30—32) were found to contain fully formed active hexacanth embryos. It would thus appear that passage to the exterior with the faeces is, under normal circumstances, the necessary condition for the development of the hooked embryo.

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EXPLANATION OF PLATES 22—24,

Illustrating Prof. William A. Haswell’s paper “On a Cestode from Cestracion.”

LIST OF REFERENCE LETTERS.

c. Cirrus. *c.s.* Cirrus sheath. *cu.* Cuticle. *d.* Depression at posterior end of free proglottis. *d.v.m.* Dorso-ventral muscular fibres. *e.c.m.* External layer of circular muscular fibres. *e.l.m.* External layer of longitudinal muscular fibres. *ex.* Main excretory vessel. *f.d.* Fertilising duct. *h.* “Head” of separate proglottis. *i.l.m.* Internal longitudinal layer of muscle. *n.c.* Nerve cord. *o.d.*¹ First part of oviduct. *o.d.*² Second part of oviduct. *ov.* Ovary. *ov.m.* Median part or isthmus of ovary. *p.u.* Ootype or primary uterus. *r.s.* Receptaculum seminis. *s.d.* Sperm duct. *s.g.* Shell-gland. *s.u.* Uterus. *sw.* “Swallowing apparatus.” *sw.m.* Muscular layer of swallowing apparatus. *sw.my.* Myoblasts of swallowing apparatus. *te.* Lobes of testis. *v.* Lobes of vitelline glands. *va.* Vagina. *v.d.* Vitelline ducts. *v.r.* Vitelline reservoir. *v.r.c.* Constriction at posterior end of vitelline reservoir. ♂. Male reproductive aperture. ♀. Female reproductive aperture.

PLATE 22.

FIG. 1.—Scolex of *Phyllobothrium vagans* magnified.

FIG. 2.—Last proglottis of strobila magnified.

FIG. 3.—Free proglottis, dorsal aspect. Nervous system, blue; excretory vessels, green; testicular ducts, red.

FIG. 4.—Portion of transverse section of strobila, showing integument and muscular layers. × 600.

FIG. 5.—Transverse section of cirrus. *cu.* Cuticle, with spinules. *c.m.* Layer of circularly arranged muscular fibres. *l.m.* Layer of longitudinal muscular fibres. *my.* Layer of myoblasts.

FIG. 6.—General view of the female reproductive apparatus as seen from the ventral side.

FIG. 7.—Dorsal view of the median part of the ovary and of the neighbouring ducts.

PLATE 23.

FIG. 8.—From a series of longitudinal (horizontal) sections. Section passing through swallowing apparatus, first part of oviduct and main vitelline duct. $\times 450$.

FIG. 9.—From the same series. Section dorsal to that represented in Fig. 8, showing vagina, receptaculum seminis, and shell-gland. $\times 450$.

FIG. 10.—From the same series. Section dorsal to that represented in Fig. 9, showing receptaculum seminis and fertilising duct. $\times 450$.

FIG. 11.—From a series of transverse sections. Section passing through swallowing apparatus and median part of ovary.

FIG. 12.—Section immediately behind that represented in Fig. 11.

FIG. 13.—From an oblique series. Mouth of swallowing apparatus. $\times 600$.

FIG. 14.—From a transverse series. Showing swallowing apparatus and its relations to ovary. $\times 600$.

FIG. 15.—From a transverse series. Showing relations of swallowing apparatus to oviduct. $\times 600$.

FIG. 16.—From a transverse series. Swallowing apparatus and oviduct. $\times 600$.

FIG. 17.—From a horizontal series. Section of oviduct at the point where the ducts of the shell-gland open into it; an ovum in the act of union with a yolk-cell.

FIG. 18.—Transverse section to show the relations of the primary uterus, the vagina, and the ruptured secondary uterus.

PLATE 24.

All the figures drawn under Zeiss's apochromatic 2.0 mm. objective and compensation ocular 12, magnifying 1100 diameters.

FIG. 19.—Egg with unsegmented ovum. From preserved specimen.

FIG. 20.—Two-celled stage. Fresh specimen, showing the globules and concentrically laminated bodies of the vitelline mass.

FIG. 21.—Two-celled stage. Preserved specimen.

FIG. 22.—Three-celled stage.

FIG. 23.—Four-celled stage.

FIG. 24.—Stage of about eight cells.

FIG. 25.—Surface view of blastoderm of a somewhat later stage than that represented in Fig. 24.

FIG. 26.—Stage of about fourteen cells.

FIG. 27.—Surface view of disc-like blastoderm.

FIGS. 28 AND 29.—Disc-like stages seen edgewise.

FIG. 30.—Hexacanth embryo with the hooks retracted.

FIG. 31.—Hexacanth embryo with the hooks everted.

FIG. 32.—Egg containing hexacanth embryo. Fresh specimen.



The Development of *Lepidosiren paradoxa*.

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Part III.—Development of the Skin and its Derivatives.

With Plates 25—28.

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INTRODUCTION.

IN the following pages I give an account of the chief results obtained from my investigation of the development

of *Lepidosiren*, in so far as they relate to the skin and certain organs associated with it. Some structures which ought logically to be described now have been deliberately omitted: such are the external gills, which I propose to consider at the same time as the branchial clefts. I also make no attempt to describe the various organs with an equal degree of fulness. In regard to some, which I have found specially interesting, I give a fairly detailed account; in regard to others I am content to outline the main features.

I have endeavoured to avoid obscuring my description by going into masses of minute detail, feeling that by so doing I should often be trespassing in regions where individual variation, and the "probable error" of observation, make results useless if not actually misleading.

THE GENERAL EPIDERMIS.

It has already been shown that the first part of the epidermis to assume a fairly definite shape is that covering the ventral surface of the embryo, which is simply the persistent roof of the segmentation cavity. The epidermis covering the dorsal surface of the body, on the other hand, develops later. It has also been shown that during gastrulation, in *Lepidosiren*, the dorsal lip of the blastopore is composed of a mass of undifferentiated cells, showing no distinction into layers. In this *Lepidosiren* differs from *Ceratodus*, where, as Semon points out, the epiblast is marked off by a distinct split right back to the lip itself. Elsewhere than at the blastopore lip epiblast is formed by delamination from the large yolk-cells underneath. By Stage 14, when the process of gastrulation is finished, the embryo is already covered uniformly by a definite stratum of epiblast composed of two layers of closely apposed flattened cells, except just in the lip of the blastopore where the germinal layers are still, and will remain for some time

undifferentiated. From the first the epiblast is thicker in the region overlying the archenteron, the cells of which it is composed being here somewhat columnar. The fate of this thickened region, which is destined to give rise to by far the most complicated product of the ectoderm, the central nervous system, may conveniently be left out of consideration until a little later.

In regard to the general ectoderm there is little change to chronicle for a considerable period. In an embryo of Stage 25 it is still two-layered, the bounding surfaces of the two layers being smooth and parallel. Prominent yolk granules are still present in the cells, and the outer layer has formed on its surface a fine but distinct cuticle. When the tail begins to form the ectoderm at the tip of this thickens, its cells assuming a cuboidal form, but remains two-layered.

The general ectoderm retains its two-layered condition for some time. Increase in thickness by division of the lower layer cells begins at a period varying from about Stage 32 to about Stage 35.

In various young *Lepidosirens*, which have been fixed in strong Flemming's solution, I have been able to make out that certain of the ectodermal cells are provided with tail-like processes of cell substance, closely resembling the tails so characteristic of the coelenterate epithelial cells (Pl. 25, fig. 1). The tails of the ectodermal cells in *Lepidosiren* are very difficult to observe, showing up distinctly only in well-fixed material in which the cells are slightly separated from one another. They run along the inner surface of the epidermis, forming a kind of plexus-like layer. Into this layer pass also processes from the underlying mesenchyme cells, so that it forms an organic connection between ectoderm and mesenchyme.

The glandular structures of the fully formed skin of *Lepidosiren* (Pl. 25, fig. 2) are one of its most characteristic features. The tall unicellular mucus glands, which, in the adult, form a palisade-like arrangement through the whole thickness of the skin, begin to appear about Stage 35

as ordinary cells of the epidermis, whose cytoplasm assumes a clear vacuolated appearance, the whole cell remaining in form and size like its neighbours. By Stage 38 the gland-cells have become predominant by their size, and they are also elongating in shape.

The multicellular glands appear about the same time as downgrowths of the deep layer of the epidermis, and here again we find that the rudiment is solid, and the cavity appears secondarily. This we can naturally not put down, as we do in the case of certain other organs arising similarly, to any such simple cause as the presence of yolk. By Stage 38 a large cavity has appeared, but it is not yet open to the exterior.

Cement Organ.

A remarkable local development of epidermal gland-cells is afforded by the cement organ, which, as indicated before, retains through life the crescentic shape shown by Thiele to be characteristic of the organ in its early stages in Batrachians. It is a curious point, however, to which my attention was first drawn by my friend Mr. Bles, that in the Amphibia the organ is derived from the superficial layer of the epidermis, not the deep layer as in *Lepidosiren*.

The first indication of the cement organ appears about Stage 23 (Pl. 25, fig. 3 A) as a slight thickening of the deep layer of the epidermis, the superficial layer passing over it hardly affected. By Stage 25 the thickening has considerably increased, and the superficial layer now shows signs of breaking down over the middle of the gland, so that here the deep cells are exposed to the external medium (fig. 3 B). By Stage 31 the gland is fully functional. Its cells are tall and columnar with nucleus at the base, and protoplasm showing peripherally a clear transparent appearance.

During the later stages of development the glandular surface becomes involuted slightly, and at the same time its lower edge becomes tilted up somewhat, so that the organ projects conspicuously above the adjoining skin surface.

Degeneration of the Cement Organ.—The process of atrophy of the cement organ is a comparatively rapid process, taking place about Stage 35. It is illustrated by fig. 3 D.

In the early stages of degeneration the glandular epithelium becomes penetrated by vascular loops, and leucocytes begin to concentrate in its neighbourhood. At a later stage (e.g. Stage 35, fig. 3 D) there are crowds of leucocytes collected about the gland, and it is now seen that they are laden with fatty and other granules, the product of their active metabolism. The glandular part of the ectoderm becomes gradually consumed, and the adjoining epidermis becomes shrivelled and has its surface thrown into wrinkles as the gland cushion diminishes in size.

Pigment Cells.

About Stage 35 branched pigment cells begin to appear in the ectoderm. I believe that these are all mesodermic in origin. In sections from embryos of about this stage many examples of pigment-laden chromatophores may be seen in process of migration into the ectoderm.

The only case, in fact, that I have found of pigment granules being formed to any conspicuous extent in epidermal cells is that of the pigment layer of the retina.

Changes in Chromatophores caused by Alteration in the Amount of Incident Light.

I have already referred (Part I, p. 320) to the remarkable difference in the appearance of a young *Lepidosiren* during the day and night. A *Lepidosiren* of Stage 38, which by day is of a deep rich brownish black, becomes at night-time quite colourless, the change being associated with the withdrawal of the dendritic pseudopodia of the chromatophores. An inspection of Pl. 25, figs. 4 A and 4 B, will

illustrate the appearance of the skin of a *Lepidosiren* of the stage mentioned during the day and during the night. The night specimen had been exposed to faint lamp-light for several minutes, and consequently the retraction of the pseudopodia is not quite complete.

From fig. 4 it will be seen that the black chromatophores tend towards two distinct types, differing in the appearance of the contained pigment and in the degree of ramification of the pseudopodia.

In type A, which is the less numerous, the pigment is very black, the cell body is compact, and the pseudopodia are long and comparatively slightly branched, and often present a varicose appearance. In type B the contained pigment is less opaque, of a brownish colour, and the cell body is often very irregular in shape, projecting in great trunks from which arise numerous short and very irregular pseudopodia.

Of these type B appears to be the more highly sensitive to light, a much fainter amount of light sufficing to cause extrusion of its pseudopodia.

When in a state of maximum expansion the pseudopodia frequently anastomose both with their neighbours and with those of other chromatophores. Anastomosis often takes place between pseudopodia belonging to chromatophores of the two different types. This, together with the presence of intermediate forms, indicates that the two types are not really distinct, but are merely the extremes of variation of a single type.

It is instructive to compare vertical sections through the skin in different light conditions. Fig. 5 illustrates such sections from (1) a young *Lepidosiren* of Stage 38, killed at 9 p.m., by faint lamp-light (fig. 5 c), (2) one of the same brood taken from deep shade at 2 p.m. (fig. 5 b), and (3) a rather younger *Lepidosiren* taken from an open white enamelled dish with clear water and exposed to bright diffused daylight (fig. 5 a).

In (1) the chromatophores are in their state of maximum contraction, and I may mention that the scattered chromato-

phores deep down in the substance of the body are also contracted.

In (2) the chromatophores have their pseudopodia fully extruded. In the case of pigment cells within the epidermis the pseudopodia pass between the cells up towards the surface. The chromatophore tends to push its pseudopodia towards the light; their movements are positively heliotropic. In the case of chromatophores lying in the superficial layer of the dermis the cells flatten themselves out against the lower surface of the epidermis, forming with their pseudopodia a practically continuous light-proof coat.

In (3) the chromatophores are seen to have their pseudopodia at the maximum of extension.

THE "STOMODÆUM."

In the young *Lepidosiren* up to Stage 30 there is no stomodæum present; the enteric rudiment, solid in this region and sharply marked off from surrounding tissues by its cells being packed with large yolk granules, extends right up to the external epiblast.

About the stage mentioned a change is seen to be setting in in the anterior part of the enteric rudiment, corresponding to what will become the buccal cavity. The superficial layer of the still solid rudiment is seen to be approximating in character to the ectoderm. Its yolk granules become finely broken up, showing that active metabolism is taking place; protoplasm and nuclei are becoming more abundant. In this way there arises a layer of definite epithelium continuous anteriorly with the external epiblast, sharply marked off from the embryonic connective tissue outside it, but internally passing without any sharp boundary into the yolk-laden mass inside (cf. Pl. 25, fig. 6 A). It is, as it were, as if an influence were spreading inwards from the external epiblast, gradually transforming the original "endoderm" yolk-laden cells into ectoderm like itself. I find no evidence of

an actual bodily involution of ectoderm such as is ordinarily associated with the term stomodæum. On the contrary, the perfectly gradual transition between the "stomodæal" cells and the typical yolk-laden endoderm cells shows quite conclusively that the former are being derived from the latter. The buccal rudiment retains its solid character till about Stage 31. About this time the cells in its interior begin to degenerate and break down, and so give rise to the cavity of the mouth.

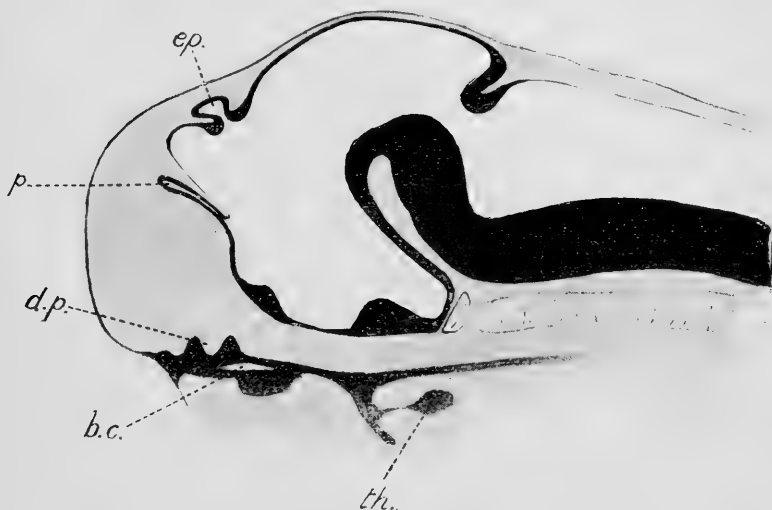
The tooth germs begin to appear long before there are any traces of lumen in the buccal cavity.¹ Already in Stage 32 they may be detected.

Development of the Teeth.

One of the most striking points brought out by Professor Semon's researches on the development of *Ceratodus* has been the way in which the so characteristic tooth plates are formed by the joining together, by dermal bony trabeculae, of originally separate denticles. On coming to consider the tooth development of *Lepidosiren* I not unnaturally expected to find a similar state of affairs, and I was accordingly much astonished on using appropriate macerating media to fail completely to discover separate denticles. I then turned to young specimens of *Ceratodus*, and had no difficulty in completely confirming Semon's description. In *Lepidosiren* the only possible reminiscence of such a stage in tooth

¹ In Urodele Amphibians the teeth similarly develop before a lumen is formed, and the lining of the buccal cavity appears to arise in them just as in *Lepidosiren* and *Protopterus*. Röse (Schwalbe's 'Morphologische Arbeiten,' Bd. iv, S. 182), in describing the development of the teeth in *Urodeles*, talks of the buccal cavity being "mit Dotterplättchen und abgestossenen schollenförmigen Epithelzellen ausgefüllt." On the contrary, I should say, from a study of my own sections of Urodele embryos (*Amblystoma* and *Triton*), that the buccal cavity has not yet arisen at the stage of which Röse is speaking. In fig. 6 B I figure a section of the mouth region of an *Amblystoma* of the stage in question for purposes of comparison with the corresponding section from *Lepidosiren*.

arrangement is to be found in the fact that in the young individual the teeth are furnished with definite prominent pointed cusps—each probably representing the tip of an originally simple denticle,—although in ontogeny they develop as a perfectly continuous ridge from the beginning.



TEXT-FIG. 1.—Sagittal section through head region of a *Protopterus* larva (Stage 33). Cam. Zeiss A*, oc. 4.

b.c. Buccal cavity. *d.p.* Dental papilla. *ep.* Pineal body.
p. Paraphysis. *th.* Thyroid rudiment.

The first obvious rudiments of teeth occur about Stage 30 in the form of a thickening of the oral epithelium,¹ under which the mesoblast becomes concentrated as it were, the nuclei being crowded much more closely together than elsewhere.

By Stage 31 the thickening is growing downwards into the mesoblast so as to border on each side a ridge-like "papilla" of mesoblast (Pl. 26, fig. 7 A).

The first traces of hard structure in the tooth appear about Stage 32, when a conical calcareous cap appears beneath the

¹ For general topographical relations of this see Text-fig. 1.

enamel organ. It is difficult to arrive at a certain opinion on the morphological nature of this first formed cap. It adheres strongly to the enamel organ, as shown by the torn surfaces when the two structures have been pulled apart in process of preparation, and in many cases it is for a time sharply marked off from the underlying dentine. On the other hand, it differs from ordinary enamel in the much larger proportion of organic matter, which causes it to remain quite distinct even in decalcified specimens.

On the whole, I am inclined to look upon this structure as being enamel, though of a somewhat modified kind.

The structure of the palatopterygoid teeth about Stage 35 may be gathered from the sections represented in figs. 7 B, 7 C, and 7 D. The enamel forms a distinct cap tapering off towards its edges, and sharply marked off from the underlying dentine. It shows a faint striation perpendicular to its surface. In undistorted sections the flat inner ends of the enamel cells abut close against it (fig. 7 D).

At this stage there is a quite definite though still thin layer of dentine lying within the enamel cap. The broadened outer ends of the odontoblasts come into close contact with one another, and form, to the eye, a quite continuous mass (fig. 7 D). As they pass into this their protoplasm shows a development of fine fibrillæ crossing one another in all directions. Traced still further out the fibrillar mass gradually takes on more and more deeply the stain which, in Heidenhain's hæmatoxylin preparations, indicates the presence of calcareous matter. The thin outer layer is, in fact, fully calcified dentine, on its inner side passing by imperceptible gradations into the ordinary protoplasm of the odontoblasts, on its outer side marked off from the enamel by a sharp boundary.

In Stage 36 (fig. 7 E) the formation both of dentine and of the bony trabeculæ which form the spongy basal support for the tooth is seen to have made considerable progress. The ridges of the tooth now approach the surface of the oral epithelium, which is becoming thin over their apices prepara-

tory to breaking through. The enamel layer is closely fused with the underlying dentine; the sharp line separating the two has completely disappeared, and it is only possible, by the use of very high powers, to distinguish the enamel by its clear appearance without any obvious structure from the dentine, which still shows a faint reticular or fibrillar structure—the remnants of the more obvious structure of the same kind in the uncalcified odontoblast.

Finally, in Stage 38 (fig. 7 F), when the young *Lepidosiren* has already begun to feed, the teeth have broken freely through the oral epithelium, the enamel organ having disappeared entirely except for a vestigial flap (fig. 7 F, *e. o.*) sticking up all round the base of the tooth. The enamel is now no longer to be detected in my sections: it has probably been worn off, being doubtless, from its larger proportion of organic matter, much less hard than ordinary enamel. The mass of dentine has much increased in size. A little later its central portion assumes the hard glassy character of the vitrodentine of the adult ("Enamel," Tomes' 'Dental Anatomy,' fifth edition, p. 263).

HYPOPHYSIS.

The hypophysis is somewhat obscure in *Lepidosiren*.

In Stage 23+ it is visible as a somewhat wedge-shaped ingrowth of the deep layer of the epiblast.

In Stage 29+ the deep end of the structure has become slightly swollen, with indications of a longitudinal split in its middle; the portion connecting this with the ectoderm is thinned down to a narrow thread occupying the space between the closely approximated front end of the gut and the floor of the fore-brain.

At a stage very slightly later (30) the connecting isthmus is nipped through, while the expanded extremity, whose split is now widening out into a definite cavity, lies as a closed sac beneath the infundibulum.

Eventually the hypophysis becomes here as elsewhere closely united with the infundibulum, its dorsal portion becoming partly penetrated by tubular outgrowths of the latter (saccus vasculosus, cf. p. 432, Text-fig. 2, H).

CENTRAL NERVOUS SYSTEM.

As the brain of the adult *Lepidosiren*¹ has never before been investigated in the fresh condition, I give on Plates 26 and 27 figures illustrating its conformation, and showing the roots of the cranial nerves, including those of the fourth and sixth, whose existence in *Lepidosiren* has hitherto been doubted. By a comparison with Burekhardt's figures of *Protopterus* it will be seen that the two brains are very similar. In dorsal view the only difference is in the relative size of the different parts. In *Lepidosiren* the mid-brain region is relatively longer, the thalamencephalon relatively shorter than in *Protopterus*. In my figure I have not shown the extensive system of outgrowths from the saccus endolymphaticus which here, as in *Protopterus*, overlies and to a great extent hides the region of the hind brain.

In the ventral view of the brain the cerebral hemispheres are not sharply marked off from the thalamencephalon. The swelling at the base of the olfactory nerves is much more conspicuous, owing to the smaller size of the post-olfactory lobe which underlies them. The lobi inferiores are much more strongly developed, the hypophysis is more rounded in form, and the hind brain is like the cerebral region less broad from side to side as compared with the thalamencephalon.

In the side view of the brain the most striking difference

¹ In dissecting the brain of *Lepidosiren* one is struck by the extraordinary development of richly ramifying blood-vessels within the cranial cavity, forming a packing all round the brain. This may possibly be an adaptation to the times at which it is impossible to make the blood rich in oxygen, during the final stages in drying up of the swamps, or during casual rainfalls in the dry season.

from what is found in *Protopterus* occurs in the cerebral hemisphere in the much smaller development of the post-olfactory lobe. The "lobus hippocampi" described by Burckhardt for *Protopterus* is less distinctly marked off in *Lepidosiren*, but is still distinctly visible.

The Development of the Main Topographical Features of the Central Nervous System.

I now proceed to describe in outline the main features in the brain and spinal cord of *Lepidosiren*. The minute structure and details of histogenesis I propose in this general account of the development to leave completely on one side.

On Pl. 27, fig. 10, are given a series of drawings of the brain in side view, and on Pl. 26, fig. 8, are given selected stages as seen from the dorsal aspect, and an inspection of these figures will suffice to give a clear idea of the evolution of the external features of the brain without any elaborate verbal description.

In my description of the early stages in development the brain and spinal cord were left (this *Journal*, vol. 45, p. 23) when they were still in the condition of a partly solid rudiment. From the beginning the anterior or brain region is distinguished by its greater width.

At about Stage 20 or 21 a slight constriction appears marking off the region of the hind brain from the region in front of it.

At about Stage 25 a transverse wrinkle in the floor of the brain begins to appear to mark the commencement of cranial flexure. By Stage 26 (fig. 10 A) this has become well marked. A slight bulging on each side of the thalamencephalon at this stage marks the rudiment of the cerebral hemisphere.

By Stage 29 (cf. fig. 10 B) the cranial flexure has become more pronounced, and a depression of the brain-roof has begun to show itself in the region of the anterior limit of the hind brain. The anterior corner of the hind brain has grown

out to form a prominent knob on each side, and the cerebral hemisphere has become more distinct.

From Stage 30 to 32 (figs. 10 c, d, and e) the chief changes consist in the close approximation of the infundibular region to the floor of the hind brain, in the appearance of the pineal outgrowth, and in the commencing forward growth of the two hemispheres. It is to be noted that up to nearly Stage 32 there is no obvious separation of mid-brain from thalamencephalon. In a brain of about Stage 35 (fig. 10 f) the chief advance consists in the considerable growth forwards of the hind brain on each side, so as in side view to completely hide the floor of the mid-brain. The roof of the thalamencephalon, forming for the most part the pineal cushion,¹ is now quite sharply marked off from the roof of the mid-brain. From now onwards to Stage 38 changes in external conformation are but slight, as will be seen from fig. 10 g, the chief one being in the upward growth of the hemisphere-roof, so that it, with the pineal cushion, rises to about the same horizontal line as the summit of the mesencephalon.

In the last stage (Stage 39), which I figure (fig. 10 h), a marked advance towards the adult condition is seen, the cerebral hemispheres having undergone a large increase in antero-posterior length. The olfactory lobe is already marked off.

The chief change subsequent to this consists in the further great elongation of the brain axis.

Dorsal Aspect of the Brain.

The earliest stage which I have figured (fig. 8 A, Stage 31) illustrates (1) the relatively enormous size of the hind brain; (2) the fact that mesencephalon and thalamencephalon form a single perfectly definite brain region, on the roof of which the pineal body has appeared; and (3) the paired independent rudiments of the hemispheres.

In the view of Stage 35 (Pl. 26, fig. 8 B) the forward

¹ Zirbelpolster.

growth of the hind brain on each side is seen, the elongation of the mesothalamencephalic region and its distinct division into a mesencephalic part behind, and a thalamencephalic portion in front, the latter thin-roofed, and forming a kind of pillow or cushion (pineal cushion), upon which the pineal body rests. Lastly, the elongation of the cerebral hemispheres has now begun.

In the brain of Stage 38 (fig. 8 c) this elongation of the cerebral hemispheres, of the mid-brain, and of the lateral angles of the hind brain is seen to have gone on still further.

Finally, in the adult brain (fig. 8 d) the great elongation of the antero-posterior axis is very obvious, affecting all regions of the brain except the thalamencephalon. It will be noticed that the lateral angles of the hind brain have lagged behind in this lengthening, so that now they do not project forwards at all. A curious shifting of the point of origin of the olfactory nerves is also seen to have taken place in the later stages of development, the point of origin in the adult being terminal and anterior instead of external and some distance back from the front end of the hemisphere.

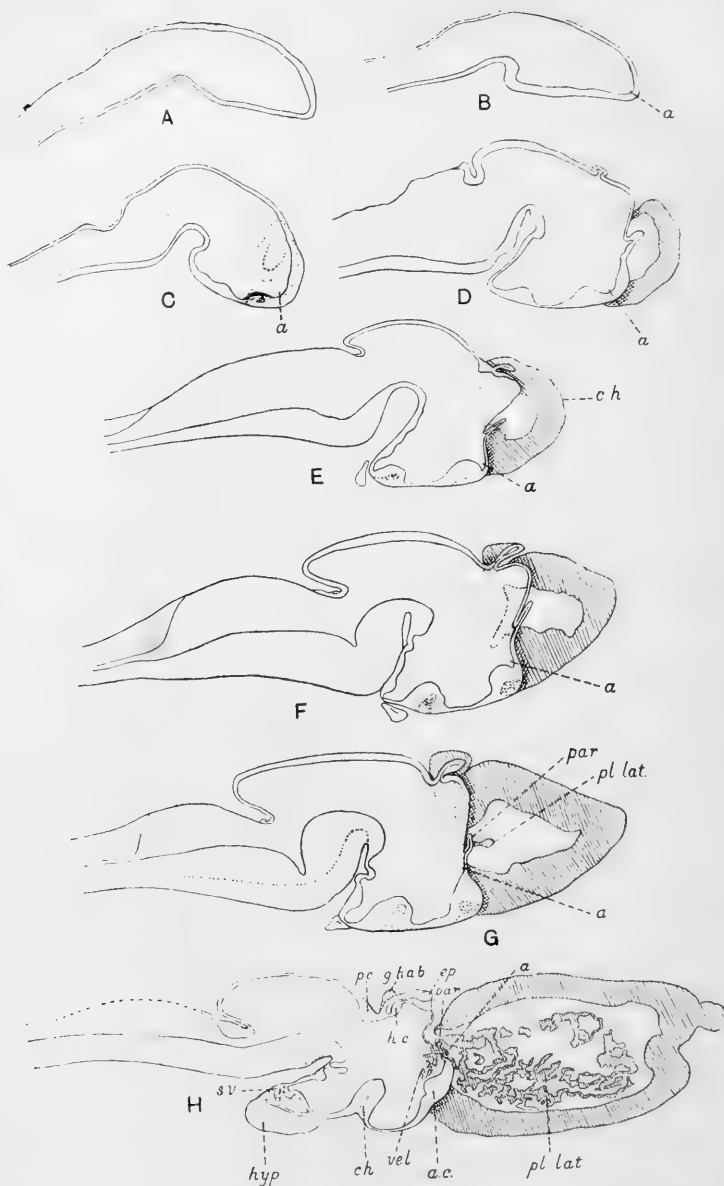
Changes in the Sagittal Section of the Brain during Development.

It is very instructive to compare sagittal sections of the brain taken at various stages of development, and in Text-fig. 2 I give figures of a series of such sections. The outlines of the sections are from camera drawings.

In Stage 25 (fig. A) a slight elevation of the brain floor is seen; this marks the hinder boundary of the thalamencephalon, and the portion in front of it corresponds with what will later on be the floor and part of the anterior wall of the thalamencephalon.

At Stage 28 (fig. B) the posterior boundary of the thalamencephalon is more strongly marked: the floor of this part of the brain shows a slight thickening.

Within the next two stages (cf. fig. C) considerable



TEXT-FIG. 2.—Camera tracings of sagittal¹ sections through the brain of *Lepidosiren* at successive periods of development. Figs. A—G are drawn under the same magnification, Fig. H under a lower magnification. A stage 25, B stage 28, C stage 29, D stage 31, E stage 31, F stage 35, G stage 38, H adult in second year.

Reference Letters: a. Anterior end of floor of brain rudiment. a.c. Anterior commissure. c.h. Cerebral hemisphere. ch. Optic chiasma. ep. Pineal body. g.hab. Ganglion habenulae. h.c. Habenular (superior) commissure. hyp. Hypophysis cerebri. par. Paraphysis. p.c. Posterior commissure. pl. lat. Choroid plexus of lateral ventricle. s.v. Saccus vasculosus. vel. Plexus of third ventricle.

Structures occurring not in the sagittal plane, but in sections parallel to but some distance from it, are shaded with oblique lines.

developments take place. The fold in the brain floor behind the thalamencephalon has become still better marked, and over it the brain roof bulges upwards in what will later be the roof of the mid-brain. Posterior to this the brain-roof is distinctly thinner, foreshadowing the membranous condition of the roof of the fourth ventricle.

In the floor of the thalamencephalon a depression has appeared, separating two elevations. Of these latter the anterior one is the rudiment of the anterior commissure, the posterior of the optic chiasma, while the depression between is the preoptic recess.

Stage 31 (fig. D).—The fold of the brain floor behind the thalamencephalon has now increased very much in height; a downfolding of the roof has also appeared, bounding the mid-brain roof behind.

The elevations in the floor of the thalamencephalon formed by the rudiments of anterior commissure and optic chiasma are now much more strongly marked though still without fibres.¹ The pineal rudiment makes its appearance about this time as a small simple diverticulum of the brain roof, and in fact the thalamencephalon has assumed very much its definitive character.

The anterior wall of the thalamencephalon at about the middle of its height is prolonged upwards and forwards as a cylindrical tube passing between the hemispheres, and ending blindly by a rounded end in front. This must be the paraphysis, and it closely resembles the paraphysis of *Urodeles*.²

Stage 31 + (fig. E).—Fibres have now appeared in the chiasma and anterior commissure. The front wall of the thalamencephalon is thinning out. The roof of mid-brain and thalamencephalon is of practically uniform thickness throughout, and there is no definite distinction between the two. Both the superior and the posterior commissures are well

¹ In regard to the region of the thalamencephalon, I propose to adopt the usage of the terms epiphysis, paraphysis, etc., favoured by Gaupp (*Anat. Hefte*, Abth. 2, Bd. vii, S. 229).

² See Minot, '*Amer. Journ. Anat.*,' vol. i, p. 93.

developed, and fibres have appeared over most of the cerebral and mid-brain surface.

The paraphysis has increased in size, forming a tube about .05 mm. in diameter, and with walls composed of a single layer of cubical cells. This runs straight forwards and upwards for a distance of about a quarter of a millimetre, and ends blindly in front.

Stage 35 (fig. F).—The main new features are the appearance of a sharp depression marking off the floor of the mid-brain from that of the hind brain, and the fact that the region of the anterior commissure is undergoing a rotation forwards and upwards, so that it is becoming transferred from the floor of the thalamencephalon to its anterior wall. By this stage the process is still inconspicuous, but later on it becomes very obvious.

The habenular commissure is very well developed. The paraphysis opens into the cavity of the thalamencephalon almost vertically under the root of the pineal body. The paraphysis has altered its position, having become rotated backwards so as to lie flat against the lamina terminalis almost vertically.

Stage 38.—The changes seen in sagittal section are comparatively trivial. The paraphysis is, however, relatively reduced, and the mid-brain is more sharply marked off from the hind brain both in roof and floor.

Development of the Various Brain Regions.

It will now be convenient to run over the main points in the development of each of the regions of the brain in turn.

Thalamencephalon.

We have already seen that the thalamencephalon begins to be marked off from the regions behind it as early as Stage 25, and it rapidly assumes its permanent features. The roof is for a long time perfectly continuous with that of

the mid-brain. The first indication of where the boundary between the two regions will come is given by the appearance of the pineal body. Appearing first about Stage 31 as a simple flattened evagination of the brain roof, and never showing at any time any symptoms of division into a true pineal body and a parietal organ, the pineal body increases somewhat in length, and eventually becomes somewhat carrot-shaped, being attached by its hinder narrower end. Its walls remain simple, and the communication of its lumen with that of the brain persists till Stage 38. In the brain of Stage 39 the lumen has become obliterated posteriorly. Anteriorly it still persists, but the cells bounding it show signs of breaking down, and the cavity is to a great extent filled with granular material apparently derived from them. At no time does the pineal body of *Lepidosiren* show any eye-like characters, and it always lies well down below the skull roof.

The roof of the thalamencephalon in front of the pineal body, while remaining thin in the median line, thickens out greatly on each side to form the habenular ganglion. These thickenings become conspicuous about Stage 31, and the commissure connecting them—the superior commissure—becomes conspicuous about the same time.

The pineal body lying on the roof of the thalamencephalon presses down the thin roof between the habenular ganglia, very much as a head does the pillow on which it rests. Consequently the name pineal cushion or pillow (*Zirbel-polster*) is a thoroughly suitable one for this region of the brain-roof. From the originally anterior wall of the thalamencephalon is developed the paraphysis, the chief characters of which have been sufficiently brought out in the study of sagittal sections. Appearing first about Stage 31 as a small evagination of the anterior wall of the thalamencephalon at about its middle, it becomes a straight tubular structure, running upwards and forwards between the hemispheres. The paraphysis soon becomes relatively reduced in size. By Stage 39 it forms an irregularly twisted tube, somewhat

dilated at its distal end, and lying between the choroid plexuses. The paraphysis does not give rise to plexus itself, although, from its very close relations with the choroid plexus on each side, it may on superficial examination appear to do so.¹

On the floor of the thalamencephalon it has already been seen that the preoptic recess becomes distinct very early, marking off the region of the chiasma from that of the anterior commissure. The tip of the infundibulum remains quite simple as far as Stage 38, but in Stage 39 it is found to have sprouted out into narrow, tubular, and apparently glandular diverticula, which, winding hither and thither, fuse with and to a certain extent penetrate the dorsal part of the hypophysis, forming the so-called nervous part of the hypophysis.

I must again draw attention to the curious differential growth in the anterior part of the floor of the thalamencephalon, which leads to a migration forwards and upwards of the anterior commissure, so that this structure, which at first lay on the floor of the thalamencephalon, comes to be situated well up on its anterior wall. The anterior commissure by the early appearance of its rudiment performs an important service, for it acts as a landmark to indicate the position of the anterior end of the longitudinal axis of the base of the original brain rudiment, which lies just in front of it. From this follows the important consequence that the front termination of this original axis lies in the adult *Lepidosiren*, by no means at the tip of the infundibulum, but high up on the anterior wall of the thalamencephalon.

It will have excited attention that I have, in treating of sagittal sections, said nothing about the velum. The velum is in fact a paired structure. In accurately mesial sections the anterior wall of the thalamencephalon is as shown in the figures, but upon either side of the mesial plane

¹ In this I entirely agree with Minot, *op. cit.*, p. 95.

and just posterior to the paraphysis it becomes invaginated into the third ventricle, forming a definite complicated velum, continuous on each side with the choroid ingrowth of the hemisphere. The velum develops late, but is present in Stage 39.

The Hemispheres.

The high development of the cerebral hemispheres, and in particular of the pallium, forms one of the most interesting features of the brain of Dipnoans. At their first appearance the hemispheres form paired separate bulgings of the lateral walls of the thalamencephalon. There is no forward growth of the anterior median portion of the wall. The hemispheres soon begin to grow up dorsalwards on either side of the thalamencephalon. Later on there sets in marked forward growth, and the immense antero-posterior length of the hemispheres becomes in the adult one of their most conspicuous features.

As the hemispheres grow forwards their outer and inner walls become equally thickened, except in their posterior portions. Here, where the inner wall of the hemisphere faces the thalamencephalon, it remains relatively thin. About Stage 35 a small rounded portion of this thin part of the wall bulges into the lateral ventricle. This rounded projection contains a vascular loop: it is the rudiment of the lateral plexus of the hemisphere. The rudiments of the two lateral plexuses are thus seen to be perfectly separate bilaterally symmetrical structures. The plexus rudiment at once begins to grow forwards into the cavity of the lateral ventricle. By Stage 38 it has grown about half the length of the ventricle, and has become swollen out into an irregular crumpled lamina. Finally, in Stage 39 (cf. Text-fig. 2, H), the plexus has become greatly complicated, and fills almost the whole of the ventricular cavity.

Mesencephalon.

Of the mid-brain there is little to be said. The roof remains throughout development thin in the mesial plane, except anteriorly, where it thickens out as it approaches the posterior commissure.

The floor of the mid-brain shows anteriorly very distinctly the two median pits which it has been suggested indicate the two anterior segments of the mid-brain. These depressions are seen in the series of sagittal sections (Text-fig. 2, F—H) : they first appear about Stage 35 as slight crinklings of the posterior wall of the infundibular depression.

Burckhardt points out, in regard to *Protopterus*, that the floor of the mid-brain is bounded posteriorly by inpushings of the two limiting membranes. The dorsal one of these becomes very sharply marked in *Lepidosiren* about Stage 35. It increases in depth for a time, but then flattens out again, and in Stage 39 has become replaced by a slight elevation of the surface. There appears to be no trace of the lower groove.

Rhombencephalon.

The cerebellum becomes evident about Stage 31 as a slight thickening of the roof of the fourth ventricle. The thickening is much more conspicuous laterally than in the middle line.

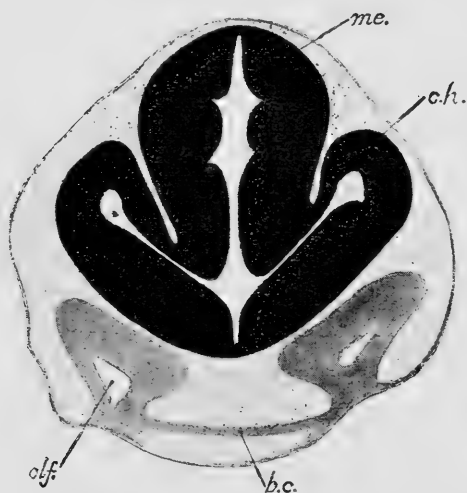
Behind this the roof of the ventricle at an early stage becomes thin and membranous.

Olfactory Organ.

The olfactory organ arises from a solid ingrowth of the deep layer of the ectoderm.

Within the few days after hatching (Stage 29 +) the cavity appears in the rudiment first in the form of a split.

This gradually increases in size (cf. Text-fig. 3), and about Stage 32 communication with the exterior is established, giving rise to the anterior nares.



TEXT-FIG. 3.—Transverse section near the anterior end of the head of a *Lepidosiren* of Stage 31. *b.c.* Rudiment of buccal cavity (still solid). *c.h.* Cerebral hemispheres. *me.* Mesencephalon. *olf.* Cavity of olfactory organ, not yet open to exterior.

The posterior nares arise later about Stage 34.

The Eye.

The rudiments of the eyes begin to develop between Stages 20 and 21 as two solid "outgrowths" from the sides of the brain rudiment (fig. 12, A).

By Stage 23 a cavity has appeared in each optic process—the fore-brain itself remaining still solid (fig. 12, B). The cavity rapidly enlarges and becomes continuous with the cavity of the fore-brain; and at the same time, by differential growth, the attachment of the optic stalk becomes carried

downwards to the ventral side of the brain (fig. 12, C). The brain being also meanwhile greatly increasing in depth, the optic rudiment is caused to slope upwards to reach its point of contact with the skin.

Later still, with the active growth in length of the brain rudiment, the point of attachment of the optic stalk becomes carried far forwards from the level at which its end portion is, so to speak, tacked on to the skin. The optic nerve now passes from its origin backwards to a great extent before reaching the skin.

Already in Stage 25 the wall of the optic vesicle lying next the skin begins to show a thickening to form the retina (fig. 12, D), and at the same time, or very slightly later (fig. 12, F), a slight thickening in the external epidermis foreshadows the formation of the lens. The retina rapidly thickens (fig. 12, G), assumes a convex surface internally, and by Stage 30 fits closely to the surface of the inner wall of the vesicle (fig. 12, H).

The lens, at first a simple thickening of the deep layer of the epiblast, begins to develop a cavity in its interior about Stage 30 (fig. 12, H). It develops somewhat ventrally to the optic vesicle, and there is at first a wide space between it and the ventral rim of the cup.

As usual the invagination takes place partly from below, and in Stage 30 there is a wide choroid fissure, involving, however, only the retinal cup. The fissure closes remarkably soon in *Lepidosiren*. Already by Stage 31 it has been obliterated.

In embryos a little older than Stage 31 I find traces only persisting; in one case a depression in the rim of the cup, in another mesoblast perforating its wall. The choroid fissure being of such a transitory nature is by no means so conspicuous a feature as we are accustomed to in the higher vertebrates.

The development of the lens from Stage 31 onwards seems to pursue a normal course, which is sufficiently indicated by fig. 12, I, J, etc.

Optic Nerve.

Lepidosiren is not well suited for a study of the histogeny of the optic nerve on account of its very slender character.

Structure of the Wall of the Optic Cup.

It will be convenient, before describing the histogenesis of the retina, to give a short account of its structure in a young *Lepidosiren* 78 mm. in length.

Near its centre the retina, including the pigment layer, measures about $125\ \mu$ in thickness.

The retina consists of three definite layers of cell elements—a layer of rod elements or percipient cells, a middle mass of nerve-cells, and a single layer of ganglion cells next the vitreous humour. These cellular layers are separated as usual by two “molecular” layers composed of the extremely fine ultimate ramifications of the cell bodies. On its inner surface the ganglion layer is covered by the thin layer of fibres going to the optic nerve.

Layer of Visual Cells.

Drawings of rod elements highly magnified are given in fig. 11, M and N.

Each cell is cylindrical in shape; at its inner end it is continued by irregular processes into the outer molecular layer, while its outer end bears the rod. Each rod is cylindrical, or rather very slightly conical. It measures from $\cdot 011$ to $\cdot 015$ mm. in length, i.e. it is much shorter than the corresponding structures in *Amphibia*. At its outer end the rod is rounded. The substance of the rod shows a division into alternate dim and clear layers, the latter being the narrower. I have not observed any longitudinal markings.

The above measurements refer to a specimen killed during daylight. Rods from the corresponding part of the retina in a

young *Lepidosiren* of the same brood, kept under identical conditions, but killed at 9 p.m., measured from .018 mm. to .028 mm. in length, the thickness being correspondingly diminished. The difference is seen on comparing fig. 11, M and N.

The rod rests on the outer end of the visual cell. The inner moiety of the cell is almost completely occupied by the ellipsoidal nucleus. This has a very definite appearance, which marks it off from the nuclei of the other layers, inasmuch as the chromatin is collected in large rounded masses, so as to give the nucleus a very coarsely granular appearance. The chromatin network, of which the rounded masses are the greatly thickened nodes, extends equally all through the interior of the nucleus.

The end of the cell next the rod is occupied by a large spherical vacuole containing a clear fluid. The protoplasm bounding this vacuole is very dense, and stains deeply with carmine, forming what looks like a distinct membrane with irregular thickenings.

As is not very clearly shown by fig. 11, there is associated with the elongation of the rod in darkness a remarkable diminution in the size of the vacuole—a diminution so striking as to suggest the possibility of there being an intimate mechanical connection between the two phenomena, the rod being elongated by the passage into it of fluid from the vacuole.

Sometimes the wall of the vacuole is in direct contact with the nucleus, at other times the two structures do not touch.

Between the two is a meniscus-shaped second vacuole, differing from that previously described by its contents not staining black with osmic acid.

The protoplasm of the cell-body forms a thin cylindrical shell round vacuoles and nucleus, while beneath the nucleus it thickens out into a more considerable mass of protoplasm, irregular in shape and giving off prolongations into the outer molecular layer.

The membrana limitans externa lies immediately external to the visual cell-nuclei. Some of these nuclei do not reach the membrane, others are in contact with it and even bulge it outwards. Occasionally one is also able to make out that the membrane passes up round the principal vacuole (cf. fig. 11).

There is no differentiation of the fully formed retinal elements into rods and cones. The structure figured by Schiefferdecker for *Ceratodus* ('Arch. mik. Anat.,' Bd. xxviii, Taf. xxiv, fig. 80) is, I have no doubt, a young rod-cell which has not yet fully developed its rod.¹

Pigment Layer.—The pigment layer of the retina consists of a single stratum of cells, polygonal, very usually pentagonal, in outline as seen in surface view.

The inner portion of the cell-body is laden with melanin granules, and during the exposure to light this is extended in a multitude of fine thread-like processes which pass up between the rods and rod vacuoles, many of them reaching to the membrana limitans externa.

In a specimen of the same brood killed by faint lamplight during the night the processes were somewhat shorter, more clumped together, and more pigment was visible in the general protoplasm of the cell-body.

I shall now proceed to describe the features which I have been able to make out in the histogenesis of the retina, more especially in regard to the development of the layer of visual cells.²

Histological Evolution of the Optic Cup.

Histological differentiation of the optic cup becomes evident about Stage 31. At this stage pigment granules are seen in the posterior wall of the cup, appearing in each cell along the

¹ Cf. for frog, Bernard, this Journal, vol. 43, page 30.

² For the most modern contribution to this subject see Levi, "Osservazioni sullo sviluppo dei coni e bastoncelli della Retine degli Urodeli," 'Lo Sperimentale,' anno liv, 1900.

face turned towards the lens, as well as in the part of each cell which adjoins its neighbour. The nucleus occupies the greater part of the body of each cell, and the part of the cell behind it is free from pigment.

In the retina itself the commencement of differentiation is seen in the fact that its central and inner portion is becoming free from nuclei, the cell-bodies in this region being composed of clear transparent protoplasm.

The development of the rods is the last important thing to take place in the development of the retina. The precise period of their appearance is, however, extraordinarily variable. While as a rule they do not appear till about Stage 35, I have met with them at least partially developed as early as Stage 32.

The appearance of the rod is heralded by the development here and there in the protoplasm of a visual cell of a small spherical droplet apparently of a fatty nature, staining black with osmic acid. As a rule these appear about Stage 35.

In the case of a visual cell which is about to develop a rod, the fatty droplet just mentioned increases in size, and comes into close contact with the external limiting membrane. As the droplet increases in size the membrana limitans becomes pushed out over it to form a hemispherical bulging. This bulges out more and more, until eventually it forms a deep pocket in which lies the fatty vacuole and a small quantity of protoplasm round it. As the vacuole continues to increase in size after it has passed into the pocket the latter often assumes a pear shape, being narrower at its base of attachment to the limiting membrane. This is shown in fig. 11, J to L.

The nucleus may project more or less into the pocket, and its peripheral end squeezing against the vacuole often pushes in its inner wall, so as to convert the sphere into a concavo-convex lens.

The rudiment of the rod appears as a conical projection of protoplasm beyond the vacuole. This condition is illustrated by fig. 1, J. The cuticular boundary of the rod rudi-

ment (i. e. the limiting membrane covering it) becomes less and less distinct, and the mass of protoplasm within becomes clear and transparent, and is converted bodily into the rod. Very soon traces of alternate dim and clear zones appear (fig. L). At first these are only two or three in number, but they increase in number as the rod increases in length.

In the completely developed rod, as already shown, the alternating dim and clear discs are very numerous, and the limiting membrane is no longer to be detected ensheathing the rod. It is, I believe, quite correct to say that now the whole of the protoplasm at the peripheral end of the visual cell has become converted into a cuticular product, the rod.

The rods develop first in the region of the axis of the eye, from which region their development gradually spreads outwards.

Auditory Organ.

The auditory organ appears as a solid ingrowth of the deep layer of epiblast. A split is already present almost from the first in its interior, e. g. Stage 21, and about Stage 23 this dilates at its inner end into a definite cavity.

The Peripheral Nerves.

The development of the peripheral portions of the nervous system is of such interest and importance that I propose to devote a separate paper to its description. I may here simply summarise the chief facts which I have observed in regard to it in *Lepidosiren*.

The first and the all-important fact which has to be mentioned is that I find that the motor nerve connection between neural rudiment and myotome already exists at a time when these structures are in close apposition. Where the myotome and spinal cord remain in contact in the section it is of course practically impossible to demonstrate the existence of the nervous bridge between them. If, however, such an embryo be examined as that figured on Pl. 10, fig. 24, of the first part

of this work¹—which has been excised from the egg and laid out in one plane while still alive, and then treated with the fixing agent, it is found that the myotomes have been pulled slightly away from the neural cord, and now the nerve-root is quite visible (cf. fig. 12 A) as a pale strand passing from the neural cord near its ventro-lateral angle, outwards to the inner surface of the myotome. At this period of development (Stage 24) there are scarcely any mesenchyma cells between myotome and spinal cord. Where occasionally such a cell appears it is heavily laden with yolk granules, and is by that easily distinguishable from the pale nerve-root.

As development progresses the myotome recedes from the neural canal with the development of mesenchymatous connective tissue between the two, and the nerve-trunk is correspondingly stretched out, or, to put it more correctly, it grows in length accordingly.

By Stage 27 (fig. 12 B) the nerve-trunk shows a very distinct fibrillation, and at its outer end its fibrils radiate out in a beautiful cone-like manner over the inner face of the myotome. In the middle of the cone the fibres are seen to pass directly into the protoplasm, and the whole arrangement of nervous tails to the muscle-cells, the latter having at this stage developed contractile fibrils in the protoplasm adjoining their three surfaces, dorsal, ventral, and external, irresistibly calls to mind the nerve-muscle arrangements of the nematode worms. Outside the conical arrangement of fibres mentioned I have not been able to demonstrate the connection of muscle-cell with nerve-root, though of course there can be little doubt that a similar relation holds. In all probability every muscle-cell in the myotome passes at its inner end into a nervous tail, but these are only conspicuous where they pass away from the inner surface of the myotome at a considerable angle; over the greater part of the surface they are lying closely apposed to it, and are hence difficult to see.

As the mesenchymatous reticulum increases in quantity between the myotome and the spinal cord its protoplasm (and

¹ 'Phil. Trans.,' vol. xcii, B.

nuclei) tends to concentrate round the nerve-trunk, so that the latter now (fig. 12 c) lies embedded in a thick strand of the reticulum. The protoplasm forming this contains yolk granules, and doubtless serves to supply the nerve-trunk with nourishment. The nerve-trunk is in need of this, for it is growing actively, both in length—as the muscle-cells are pushed farther and farther away from the spinal cord—and also in thickness. Whereas at Stage 24 the nerve-trunk measured about $1\ \mu$ in diameter, now it measures about $5\ \mu$. As the fibrils composing it do not appear to have undergone a corresponding thickening they must have increased in number. As to how this increase takes place, whether by splitting of original fibres or by formation of new fibres by the surrounding protoplasm, it is obviously impossible to make definite assertions, based merely on observation, which shall be at all reliable. Theory, of course, would favour the first view.

As regards the sensory nerves, I do not propose to say anything at present, except that my evidence, so far as it goes, points to exactly similar processes taking part in their development as I have just outlined in regard to the motor nerves.

GENERAL REMARKS.

Effect of Light upon the Chromatophores.

It will be possible from the present paper to form a clearer idea of the process of change in the chromatophores to which I alluded in my description of the external features. I still hold to the view that we have to deal with an actual retraction of pseudopodia, and not a mere movement of pigment granules into the body of the cell as held by Ballowitz. Stray pigment granules may be observed frequently remaining where the pseudopodia have been. I take these not as indicating that the cell processes are still protruded, but as having been left behind by the pseudopodia

during their retraction, after the manner familiar to any one who has watched living Foraminifera.

Circumstances, unfortunately, did not allow of my making any experiments as to whether a nervous reflex plays any part in the contraction of the chromatophores. I am inclined to think, however, that we have to do with a direct action of light upon the individual cell.

I wish to draw attention to one point; that there is a fundamental similarity in the reaction to light stimulus of the general chromatophores of the body and the cells of the pigment layer of the retina. In both we have to do with relatively slightly differentiated cells, and in both their reaction to light (in addition probably to the formation of the pigment itself) consists in their pushing out positively heliotropic pseudopodia.

General Morphology of the Fore-brain Region.

It is fashionable to accept the view that the "secondary fore-brain" is fundamentally an unpaired structure, forming topographically the anterior end of the cerebro-spinal axis of the adult—that it is a "telencephalon."

It will be seen that the conditions holding in the ontogeny of *Lepidosiren* go completely against this view. On the contrary, they support the opposite and older view, the view held by von Baer, Reichert, Goethe, and in recent times including as one of its chief exponents Studnička, that the hemispheres are fundamentally paired bulgings outwards of the lateral wall of the thalamencephalon, of a nature roughly comparable with the outward bulgings which give rise to the eyes. The portion of brain from which they project outwards is simply the anterior portion of the original fore-brain,¹ which, as has been shown, becomes marked off at a very early period of development by an upward bend in its floor,—that is to say, it is simply and solely thalamencephalon.

¹ *Vorbirn* of Kupffer.

Studnicka has recently asserted that "Der Vorderhirn aller Cranioten ist paarig angelegt."¹ *Lepidosiren* furnishes nothing to throw doubt on this generalisation, and the general tendency of modern research, especially upon forms in which the topographical relations are not liable to be distorted by the presence of a large mass of yolk with its accompanying exaggerated cerebral flexure, seems on the whole to support it. If true it ought to involve the rejection of His's brain regions telencephalon and diencephalon, as not representing true morphological entities. The old expressions thalamencephalon and prosencephalon are much more accurate, but it seems questionable whether it would not be better to drop the latter name and merely use the descriptive term "hemispheres," as this conveys no erroneous suggestion that we have to do with anything more than a local hypertrophy of the thalamencephalon wall upon each side.

The enormous importance of the primitive fore-brain—that brain region which assumes its definitive characters so extraordinarily early in development, and which appears to be already marked off in *Amphioxus*,² can hardly be exaggerated. Compared with it the hemispheres, however important they may be physiologically, are morphologically of relatively little account.

It appears to me, biassed possibly by my observations of what happens in *Lepidosiren*, that too much morphological weight is attached to the velum. It is looked upon as being the landmark which indicates in the brain roof the boundary between two important brain regions, and structures lying anterior to it are regarded as belonging to the secondary fore-brain. In *Lepidosiren* we have seen that the velum is a paired structure, also that it arises simply as a prolongation backwards from each lateral plexus. The evidence of *Lepidosiren* pointing towards the secondary fore-brain being primitively paired and to the lateral plexus being similarly

¹ 'S. B. Böhmisch. Ges.,' 1896, xv.

² Kupffer, 'Studien zur vergl. Entwicklungsgeschichte des Kopfes der Cranioten,' Heft 1, S. 71.

paired structures, it seems probable that in the paired nature of the velum we have also to do with a primitive character.

It is also seen from the study of sagittal sections through the brain at succeeding stages of development that the unpaired portion of the brain lying anterior to the level at which the velum will later appear is nothing more than the anterior portion of the thalamencephalon. It follows from this that the paraphysis does not belong to the secondary fore-brain at all;¹ it is a projection of the anterior wall of the original thalamencephalon.

Regarding speculations as to the original nature of the paraphysis, *Lepidosiren* seems to offer little definite evidence. On the whole, however, its topographical relations in *Urodeles* and *Dipnoans* suggest that it is a part of the brain wall which has been drawn out by the interposition of mesenchyme between skin and brain, through its outer end being attached to the skin; in other words, that it probably represents either an ancient sense-organ (*Selenka*) or a part of the primitive connection of the brain with the outer skin.

There has been considerable discussion as to the nature of the smaller, more ventrally situated of the two vesicles found in the pineal region of *Petromyzon*. The relations of the paraphysis of *Lepidosiren*, about whose homology there can be, I imagine, no doubt, support the view expressed by *Kupffer*² that the vesicle referred to is also morphologically the paraphysis. If this homology hold, the discovery by *Retzius* of nerve-fibres in the structure in question in *Petromyzon* would, as *Gaupp* points out, support the idea of the at one time sensory nature of the paraphysis.

The Peripheral Nerves.

It will, I think, be admitted that the facts which I have shortly sketched in regard to the development of the motor

¹ *Gaupp*, op. cit., p. 229.

² Op. cit., Heft 2, S. 10.

nerves have considerable bearing upon some not unimportant questions of general morphology.

1. I have shown that those muscle-cells of the myotome whose investigation is easy are already in organic connection with the nervous system while they lie in close apposition to it. As these muscle-cells are carried further and further away from the nerve-centre by the interposition of mesenchyme, and by the action of differential growth, they trail behind them the ever-lengthening nervous strand,¹ and there is therefore no question about calling in—so far as regards these particular muscle-cells—either a growth outwards towards them of freely ending nerve-trunks or their provision with nerves through the conversion into nerve of other tissue. They are connected while still close to the nerve-centre with a potentially nervous bridge, and as development goes on this merely increases in length and thickness.

That this holds I have been able to definitely establish only in the case of certain muscle-cells in the region of the myotome nearest the nerve-root, but it is extremely unlikely that motor nerves do not develop all according to the same general plan. They probably all develop just as do those which I have described.

2. I have shown that in the young *Lepidosiren* there exists in the myotome a neuro-muscular apparatus of an extraordinarily primitive character—a simple though enlarged epithelial cell with contractile fibres developed in the peripheral regions of its protoplasm, and with its cell substance passing out at its inner end into a kind of tail which is continued along the nerve rudiment, doubtless to pass—though this would be very difficult to demonstrate—at its central end into a nerve-cell in the neural tube.

The full consideration of muscle development will come in a later part of my work, but even in passing I think we

¹ This of course explains quite clearly the meaning of the rectilinear course of young nerves, upon which His and others lay stress. Cf. 'Arch. Anat. Phys.,' 1887, S. 375.

must recognise in the transient phase which I have described the indication of a far back phylogenetic stage in the history of vertebrate muscle.

The facts which I have summarised above in regard to the development of nerve and muscle in *Lepidosiren* support, on the whole, the theoretical views of Hensen in regard to the development of nerve, and are totally opposed to the views of Bidder and Kupffer and of His that the nerves grow out towards their motor end structures, and unite with them secondarily. They also lend additional support to the view which Sedgwick has consistently taught, and which has been supported by much recent work, such as above all that of Apáthy upon the structure of the nervous system, that we must regard the vertebrate individual in the first place as an organic and continuous whole, whose various regions are linked up in organic continuity, rather than as an aggregation of separate cells or organs.

SUMMARY OF THE MORE IMPORTANT FACTS DESCRIBED.

1. Certain of the ectoderm cells are provided with tail-like processes which pass into a subepidermal layer, into which also pass processes of the underlying mesenchyme cells.

2. The flask-shaped glands of the skin arise as solid thickenings of the deep layer of the epidermis which develop a lumen later.

3. The cement organ arises as a thickening of the deep layer of the ectoderm over which the superficial layer degenerates and disappears. Its atrophy is caused mainly by the action of phagocytes.

4. The chromatophores of the skin are mesodermal in origin.

5. There is no invagination of ectoderm to form a true stomodæum. The epithelium of the buccal cavity is developed in situ from the outer layer of the solid anterior portion of the yolk-laden enteric rudiment.

6. The tooth-plates of *Lepidosiren* are not at any stage represented by numerous separate denticles as is the case with *Ceratodus*.

7. The tooth germs appear while the mouth region is still without a lumen. They develop according to the "Placoid" type.

8. There is a well-developed enamel organ, and a layer of what appears to be a peculiar type of enamel is the first of the hard parts of the tooth to be laid down.

9. The dentine appears to arise by metamorphosis of the peripheral portions of the cell-substance of the odontoblasts.

10. The brain of the adult *Lepidosiren* closely resembles that of *Protopterus*, differing only in details. The fourth and sixth cranial nerves are present though extremely thin.

11. The thalamencephalon and mesencephalon do not become marked off from one another until relatively late.

12. The cerebral hemispheres arise as two separate lateral bulgings of the wall of the thalamencephalon.

13. The choroid plexuses of the lateral ventricles are similarly paired originally; the plexus of the third ventricle arises from them and is also paired. There is no velum in the middle line.

14. The pineal organ is simple, without any trace of a separation of a part of it to form a "parietal organ."

15. There is a well-developed paraphysis closely resembling that of *Urodele* Amphibians.

16. The paraphysis is a product of the thalamencephalon, not of the secondary fore-brain.

17. The point in the brain of the adult which corresponds to the anterior end of the floor of the neural rudiment of the embryo, lies well up on the anterior wall of the thalamencephalon just below the root of the paraphysis.

18. The rudiments of the eyes, like those of nose and ear, are at first solid, the cavity of the optic vesicle arising secondarily, rather before the ventricle of the brain at its level has appeared.

19. The lens also arises by a solid rudiment from the deep layer of the ectoderm, its cavity arising secondarily.

20. In the histogenesis of the visual layer of the retina the development of the rods is preceded by the formation of the oil globules.

21. Each oil globule passes with surrounding protoplasm into a pocket-like projection of the external limiting membrane.

22. The rod is formed directly from the protoplasm lying in the conical apical part of the pocket.

23. The motor nerve-trunks are in *Lepidosiren* already laid down at a period when myotome and neural tube are still in close apposition. As development proceeds and the myotome recedes from the spinal cord the nerve-trunk lengthens out, increases in thickness, and becomes ensheathed in mesenchymatous protoplasm.

24. In the earliest stage observed the motor nerve-trunk is seen to be perfectly continuous with the protoplasm of the, at this stage, simple epithelial muscle-cell of the myotome.

EXPLANATION OF PLATES 25—28,

Illustrating Professor Graham Kerr's paper on "The Development of *Lepidosiren paradoxa*," Part III.

The figures of sections have been drawn with the Abbe camera under Zeiss objectives a*, A, D, 3 mm. dry apochromatic, and $\frac{1}{18}$ in. homogeneous immersion. The figures of the entire brain have been done either from actual dissections, or from reconstructions by the method described by me in an earlier number of this Journal.¹

By Stage n in the descriptions of the following figures I mean the stage represented by fig. n in Part I.² Stages slightly younger or older than the figured are described as n- or n+.

¹ 'Quart. Journ. Micr. Sci.,' vol. xlv, p. 5.

² 'Phil. Trans.,' vol. xcii, p. 299.

Figures of a selected number of stages were copied in Part II (this specimen Journal, op. cit.).

I have again to express my great indebtedness to Mr. Edwin Wilson for the care which he has devoted to working up the figures.

GENERAL LIST OF REFERENCE LETTERS.

a.d. Archinephric duct. *aud.* Auditory vesicle or capsule. *a.v.* Annular vacuole. *b.* Bony trabecula. *b.c.* Buccal cavity. *b.v.* Blood-vessel. *buc.* Solid yolk-laden cells occupying position of buccal cavity. *c₁*. Chromatophore of Type A. *c₂*. Chromatophore of Type B. *c.h.* Cerebral hemisphere. *d.* Dentine. *d.g.* Dorsal root ganglion. *e.* Enamel. *e¹*. Superficial layer of ectoderm. *e²*. Deep layer of ectoderm. *e.o.* Enamel organ. *ep.* Pincal body. *f.* Fat globule. *f.l.* Fore-limb. *g.c.* Unicellular glands. *g.l.* Flask-shaped gland. *hyp.* Hypophysis cerebri. *li.* Lobus inferioris. *m.e.* Mesenchyme cells. *mes.* Mesencephalon. *m.l.* Membrana limitans. *m.n.r.* Motor nerve-trunk. *my.* Myotome. *n.* Notochord. *n.a.* Neural arch. *n.c.* Neural tube. *nu.* Nucleus. *o.* Odontoblast. *o.c.* Eye rudiment. *o.g.* Oil globule. *olf.* Olfactory capsule. *p.* Pocket. *ph.* Phagocyte. *r.* Rod. *sc.* Sclerotome. *stom.* Lining epithelium of buccal cavity arising in situ. *th.* Thalamencephalon. *v.IV.* Fourth ventricle.

The cranial nerves are indicated by the ordinary Roman numerals. In addition:—*h.m.* Hyomandibular branch. *lat.* Lateral branch. *lat.gang.* Lateral ganglion. *r.c.* Communicating branch. *s.p.* Superior palatine branch. *visc.gang.* Visceral ganglion.

PLATE 25.

FIG. 1.—Example of tailed cells from the ectoderm of a young *Lepidosiren* of Stage 35 —. Zeiss 3 mm. apochrom., oc. 2.

FIG. 2.—Section of skin from the head of an adult *Lepidosiren* to show the large unicellular glands (*g.c.*). Zeiss A, oc. 2.

FIG. 3.—Illustrating the history of the cement organ. Zeiss A, oc. 2.

A. Stage 23, showing simple thickening of the deep layer of the ectoderm.

B. Stage 25, showing the disappearance of the superficial layer over the gland rudiment.

- c. Stage 31, the gland at its full development.
- d. Stage 35, illustrating the atrophy of the gland. *b.v.* Blood-vessel;
f. Fat globules; *ph.* Phagocytes.

FIG. 4.—Portions of skin stripped off two *Lepidosirens* of Stage 38 (belonging to the same brood). Zeiss A, oc. 4.

- A. Killed at 2 p.m. in daylight.
- B. Killed at 9 p.m. in faint lamp-light.
- c.*₁ Chromatophores of Type A; *c.*₂ Chromatophores of Type B;
g.c. Unicellular gland; *gl.* Multicellular gland.

FIG. 5.—Vertical sections through the skin of young *Lepidosirens* killed under different conditions of light and darkness. Zeiss D, oc. 2.

- A. From white enamelled vessel in bright daylight.
 - B. From deep shade under a verandah.
 - C. Dropped into the fixing agent at 9 p.m. in darkness.
- [NOTE.—In A the epidermis has become pushed upwards by the action of the acetic acid in the fixing solution.]

FIG. 6.—Illustrating the formation of the lining of the buccal cavity.

- A. Part of a sagittal section of a *Lepidosiren* of Stage 30. Zeiss D, oc. 2.
- B. Part of a similar section of an embryo of *Amblystoma*, measuring 7·5 mm. in length.
buc. Still solid, yolk-laden cell-mass in position where the buccal cavity will develop later; *e*¹. Superficial layer of ectoderm; *e*². Deep layer of ectoderm; *stom.* Lining epithelium of buccal cavity arising in situ.

PLATE 26.

FIG. 7.—Illustrating the development of the teeth.

- A. Portion of a section parallel to the sagittal plane at Stage 31, showing one of the palato-ptyergoid tooth germs. The buccal cavity (*b.c.*) is beginning to form, but it is still closed anteriorly (to the right in the figure). Zeiss D, oc. 2.
- B. Portion of a similar section at Stage 35. Zeiss D, oc. 2. The figure shows the enamel cap (*e.*) and the commencing formation of dentine (*d.*). *e.o.* Enamel organ; *o.* Odontoblast. (The split round the enamel is, as shown by other sections, artificial.)

- c. Part of a transverse section of Stage 35, cutting the palato-pterygoid tooth germs longitudinally. Letters as before, and *b.v.* Blood-vessel; *b.* Bony trabeculæ. Zeiss A, oc. 4.
- d. Part of the preceding section under a higher power (Zeiss 3 mm. apochrom., oc. 2) to show the minute structure of the dentine which is being formed, and also the distinctness of the enamel layer.
- e. Part of section similar to A and B, but from Stage 36. Zeiss A, oc. 2.
- f. Part of similar section from Stage 38. Zeiss A, oc. 2.

FIG. 8.—Illustrating the brain of *Lepidosiren* as seen from the dorsal side.

- A. Stage 31+. Reconstruction Zeiss a*, oc. 2. The thalamencephalon and mesencephalon are not yet marked off from one another.
- B. Stage - 35. Reconstruction Zeiss a*, oc. 2.
- c. Stage 38. Dissection.
- d. Adult.

aud. Auditory vesicle or capsule. *c.h.* Cerebral hemispheres. *d.g.* Dorsal root ganglion. *ep.* Pineal body. *f.l.* Fore-limb. *mes.* Mesencephalon. *n.a.* Neural arch. *olf.* Olfactory capsule. *th.* Thalamencephalon (the line stops over the left habenular ganglion). *v.IV.* Roof of fourth ventricle.

CRANIAL NERVES.—I. Olfactory. II. Optic. III. Oculo-motor. IV. Pathetic. V. Trigeminal. V.¹ Ophthalmic division. V.² Maxillary division. V.³ Mandibular division. VII. Facial: VII, *h.m.* Hyomandibular; VII, *lat.* Lateral; VII, *s.p.* Superior palatine; VII and X, *r.c.* Commissure connecting lateral portions of VII and X. VIII. Auditory. IX. Glosso-pharyngeal. X. Vagus: X, *lat.* Lateral trunk; X, *lat. gang.* Lateral ganglion; X, *visc. gang.* Visceral ganglion.

FIG. 9.—View of brain of adult from below.

hyp. Hypophysis. *l.i.* Inferior lobe. VI. Abducent.

PLATE 27.

FIG. 10.—The brain of *Lepidosiren* in side view. A, B, C, D, E, represent reconstructions; F, G, H, I, dissections.

- A. Stage 26.
- B. Stage 29+.
- c. Stage 30.

- D. Stage 31.
- E. Stage 31+.
- F. Stage -35.
- G. Stage 38.
- H. Stage 39.
- I. Adult.

oc. Eye rudiment. Other letters as in Figs. 8 and 9.

FIG. 11.—Illustrating the development of the eye. All the figures illustrating the development of the eye are taken from sections transverse to the long axis of the head.

- A. Stage 21. Zeiss A, oc. 2.
th. Still solid thalamencephalon; *oc.* Eye rudiment.
- B. Stage 23. Zeiss A, oc. 2. A cavity is now arising in the optic rudiment; the brain is still solid in this region.
- C. Stage 25. Zeiss A, oc. 4. Thalamencephalon and optic vesicle are now hollow. The retinal wall of the optic vesicle is beginning to thicken.
- D. Stage 26. Zeiss A, oc. 2. Showing advanced thickening of retina.
- E. Stage 30. Zeiss A, oc. 4. Optic cup—lens rudiment with commencing cavity (*i.e.*).
- F. Stage 31. Zeiss D, oc. 2.
- G. Stage 35. Zeiss A, oc. 4.
- H. Stage 38. Zeiss A, oc. 4.

PLATE 28.

FIG. 11, I—N. Illustrating the development of the percipient elements.

- I. Stage 35. $\frac{1}{12}$ hom. imm., comp. oc. 6. Showing the formation of the fatty globules and their passage into pocket-like projections of the internal limiting membrane.
o.g. Globule; *p.* Pocket; *m.l.* Membrana limitans; *nv.* Nucleus of visual cell.
- J. Stage 35. $\frac{1}{12}$ hom. imm., comp. oc. 6. Showing first rudiment of rod as a protoplasmic projection (*r.*).
- K. Stage 35. $\frac{1}{12}$ hom. imm., comp. oc. 6. Slightly later stage of rod development.
- L. Stage 35. $\frac{1}{12}$ hom. imm., comp. oc. 6. Rod showing first signs of stratification. Appearance of annular vacuole (*a.v.*).
- M. Stage 38. $\frac{1}{12}$ hom. imm., comp. oc. 6. A fully formed rod element, fixed in the light.
- N. A similar rod element from a young *Lepidosiren* of the same age and same brood, but fixed in the dark.

FIG. 12.—Illustrating the development of the motor nerve-trunks. All three figures represent portions of sections perpendicular to the longitudinal axis of the body.

a.d. Archinephric duct; *me.* Mesenchyme cells; *m.n.r.* Motor nerve-trunk; *my.* Myotome; *n.e.* Neural tube; *n.* Notochord; *sch.* Sclerotome.

- A. Stage 24. Zeiss A, oc. 4. Section of an embryo excised from the egg and flattened out, so as to slightly separate myotome and spinal cord, and display the rudiment of the nerve-trunk which even at this stage unites the two.
- B. Stage 27. Zeiss D, oc. 2. The myotome has become separated from the neural tube by interposed mesenchyme. The nerve-trunk is lengthened out, and externally is continued into the muscle-cells of the myotome.
- C. Stage 29+. Zeiss D, oc. 2. The nerve-trunk is now ensheathed in mesenchymatous protoplasm containing nuclei and yolk granules.



The Metamorphosis of *Corystes Cassivelaunus* (Pennant).

By

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With Plates 29—31.

THE material upon which the following account of the development of *Corystes* is based has been derived entirely from tow-net collections, taken generally in or just outside Plymouth Sound, between February and May of the present year. Occurring first on February 21st, the larvæ were obtained in small numbers fairly regularly from that time onwards. Advanced stages occurred first on March 15th, and in considerable numbers. The tow-net collections of April 14th, taken in the West Channel, were remarkable for the extraordinary abundance of the zoæa stage of *Corystes*. They abounded on that date to the almost complete exclusion of all other zoæas, nearly all the specimens being at an advanced stage of development. Since April 14th the zoæas have continued to occur, but very few have been found in the early stages.

Zoæas taken in the tow-nets have been kept successfully in plunger jars for considerable lengths of time, and several have moulted into the *Megalopa* stage, the latter being kept through the next moult, either in a jar immersed in running water or in a beaker, in which the water was kept in motion by a stream of air bubbles.

Habits of the Larvæ.

The zoæa of *Corystes* is probably the same as that described by Weldon (1889), and figured as "a Portunid zoæa," though it differs from his figure in several respects. Weldon came to the conclusion that the long spines have the function of directing the movements, and enabling the animal to swim rapidly in a straight line. There can be no doubt that his conclusion is correct, for the larva always moves in the direction of the long axis of the spines. This is well shown when a zoæa is caught in an eddy in the plunger jar. It then makes a violent effort to escape, darting upwards, sideways, or straight to the bottom, according to the position of the spines at the moment. Normally the larva rises and falls in the water, swimming upwards vertically for a short distance, and then resting. In the plunger jar larvæ have a tendency to collect, especially the younger stages, at the surface against the side of the jar. Here they often push their dorsal spines through the surface film, and are hereby suspended. Sometimes they rest against the rod of the plunger, suspended in this manner, and to this habit I have owed the death of several specimens which were caught and drawn up upon the plunger rod. If a light is brought to the jar at night the zoæas become extremely active, swimming rapidly towards the light, the dorsal spine directed forwards. The body may, however, be in any position with regard to the axis of the spines, the animal swimming upon its back, side, or ventral surface.

It is of interest to note, in considering the function of the spines, that the period of their presence corresponds exactly with the period of the absence of an "auditory" organ. The latter, as Prentiss (1901) has shown, is not developed functionally till the *Megalopa* stage, when only traces of the spines remain. There is a rapid reduction in the length of the spines as compared to the total length of the body in the third and fourth larval stages, and it is in the last zoæa

that the first trace of the auditory invagination is to be found. It seems likely, then, that the spines may perform to some extent the balancing and orienting function of the auditory sac.

Besides being of balancing and directive function, the spines probably also serve as a protection. It is, of course, hard to say what are the especial enemies of the zoæas in nature. They are certainly preyed upon to some extent by *Medusæ* and *Ctenophores*, and also by each other, and in these cases the spines can be of little value. They must, however, serve as a defence against the attacks of small fish. That this is so was shown by presenting the zoæa to *Gobius ruthensparri*, a fish about 4.5 cm. long, and with a width of mouth of about 3.5 mm. In the first experiment a goby seized and rejected the zoæa six times, each time failing to swallow it. Finally the fish gave up the attempt, and the zoæa soon recovered and swam away, being, however, attacked and swallowed by a second goby after a number of failures. Other experiments showed the same thing, though the fish had no difficulty in swallowing small *Brachyurous* zoæas without great development of spines—for instance *Carcinus*.

A peculiar habit of the zoæa at all stages is that of frequently turning its abdomen backwards till the forked telson reaches and embraces the dorsal spine, scraping upwards as if to clean it. This action is so frequent that it seems not to be connected with the process of moulting, though possibly the stretching of the abdomen entailed may assist in preparing for the act. Moulting seems usually to take place at night, and must be a rapid process, for a successful moult was never observed. Zoæas were frequently found half freed from the larval skin, but these specimens never succeeded in completely freeing themselves. In fact, the new skin seems to harden so rapidly that unless the process is completed at once failure results.

The Megalopa stage is remarkably interesting from the point of view of its habits. It has most of the characters

and habits of the adult. Like the *Megalopa* of the majority of *Brachyura* it is very active, swimming rapidly by means of its pleopods, the antennæ being carried stretched straight forwards and parallel to one another, the thoracic legs bent up under the body. Unlike the zoæa it seems to be indifferent to a strong light at night, being neither attracted nor apparently repelled by it.

It does not seem to be a pelagic form properly speaking, and was only once obtained in the tow-net, and then within a fathom or so of the bottom, in deep water. Some of the specimens moulted in my plunger jars were provided with fine sand, and at once burrowed until covered completely except for the antennæ. The act of burrowing is performed just as in the adult by means of the four posterior legs, the chelipeds taking practically no part. If the sand, which must be exceedingly fine, is not deep enough to completely cover the body, the *Megalopa* pushes itself backwards till the sand is heaped up above it, often moving backwards in this way for some distance. When covered the antennæ are not necessarily held quite parallel, but the position seems to a considerable extent to depend upon the depth to which the animal has burrowed. In the buried position the respiratory current is reversed, and sets down the antennal tube, as Mr. Garstang has shown it to be in the adult. The efficiency of the antennal tube as a strainer was well shown by the sand grains resting on the interlocking hairs. One specimen of the *Megalopa*, and one of the succeeding post-larval stage, were obtained in some sand from Whitsand Bay.

As to the food of the earlier stages of the larva I have no direct observations to record. Though provided with a constant supply of the smaller plankton organisms, and with other small zoæas, I have never seen the zoæa of *Corystes* taking any food. From the appearance of the contents of the gut it seems likely that the food consists entirely of floating algæ or diatoms. I have several times seen the zoæas of other forms, such as *Eupagurus*, devouring other larvæ, but it is quite possible that the zoæas of *Corystes*, at

least in the earlier stages, are exclusively vegetable feeders. The Metazœa and Megalopa, on the other hand, have more than once been found eating zoœas, and even those of their own kind, and I have found small pieces of worm or shrimp muscle a very satisfactory food for the Megalopa.

Development of the Larva.

There appear to be four distinct stages in the development of the larva preceding the Megalopa, but I am unable to say how many moults are included in this period. The zoœas referred to the second stage differ among themselves to a certain extent in the relative development of the parts of the body, and this stage, consequently, is not very sharply separated from those preceding and following it.

First Stage (Pl. 29, fig. 1).—The measurements of the zoœa at this period are as follows, the figures given being the average of ten specimens. I have given here, as also in the stages succeeding, the minimum and maximum for each measurement.

	Average length.	Minimum and maximum lengths.
1. Length of rostrum .	1·4 mm.	1·3 to 1·5 mm.
2. „ dorsal spine	1·9 „	1·5 „ 2·0 „
3. Tip to tip of spines .	4·0 „	3·7 „ 5·1 „
4. Length of body .	2·4 „	2·3 „ 2·7 „
5. „ thorax .	·8 „	·7 „ ·85 „
6. Ratio of 4 to 3, 1 :	1·66.	

The zoœa of *Corystes* is distinguished from all other Brachyurous zoœas hitherto described by the following features:—The total length from tip to tip of the dorsal and rostral spines greatly exceeds the total length of the body. In the majority of zoœas the two measurements are approximately equal. The posterior edge of the carapace bears a fringe of short setæ. The forks of the telson have

the normal form, but bear only a single¹ lateral spine on each side. The resemblance between the zoæa of *Corystes* and that of *Thia polita* as figured by Claus (76) and Cano (91) is very striking, but the latter differs in having a much longer lateral thoracic spine, and in having two lateral spines on each fork of the telson.

The colour and its distribution in the body are also characteristic. The long dorsal spine is a rich orange colour, deepest towards the tip. The rostrum has the same colour, but the chromatophores appear to be less numerous. The labrum contains a dendritic black chromatophore, and similar chromatophores are found in the carapace, one above the mandible, and two near the postero-ventral and postero-dorsal edge of the carapace. A small orange chromatophore lies at the base of the dorsal spine. The alimentary canal is enveloped in black chromatophores, which run back along it as far as the end of the second abdominal segment. A large ramified black chromatophore lies at the joint between the third and fourth, fourth and fifth, and fifth and sixth abdominal segments. To the naked eye the liver and gut appear as a yellowish-black mass continued back as a black streak through the thorax, and the orange colour of the spines is conspicuous and distinctive.

The appendages of the zoæa are of the usual type, differing in no important respects from those of *Portunus*, for example, and do not need detailed description.

The second maxilla (Pl. 30, fig. 9) is the only cephalic appendage which calls for any remark. In it the exopodite (scaphognathite) is characterised at this stage by the possession of only five setæ, the fifth springing almost directly from the posterior edge, and not, as in *Portunus*, from the end of a narrowed prolongation of the edge.

¹ Since writing the above I have found a single specimen in the second stage of development, in which the left fork of the telson bears two lateral spines as in *Thia polita*, while the right fork bears but one. The rarity and asymmetry of this structure seems to show that its presence is due merely to an individual variation (see fig. 4).

The two pairs of maxillipedes have each a two-jointed exopodite bearing distally four long ciliated setæ.

Behind the first and second maxillipedes there are already developed rudiments of the six remaining pairs of thoracic legs. The first pair, or third maxillipedes, are longer than the rest, and bent forwards between the second maxillipedes. The fourth pair is covered by the third, and hence is not visible without dissection (see fig. 1).

As breeding females of *Corystes* are not easy to obtain, and I have consequently not been able to hatch the zoæa from the egg, it is possible that an earlier stage remains to be discovered. Still, the early development of the posterior thoracic limbs is not uncommon among Brachyurous zoæas. In many forms the third maxillipede is already marked out in the first zoæa, and in some all the thoracic appendages are visible on hatching. This is the case in *Portunus puber*, and more especially in *Inachus dorsetensis*, where even the pleopods are distinctly traceable.

The abdomen, in the first stage of *Corystes*, consists as usual of five distinct segments, the second bearing a forwardly curved process on either side. The second, third, fourth, and fifth segments each bear a short hair on their posterior dorsal edge on either side of the middle line.

The telson (fig. 2), with which the sixth segment is united, has the usual forked shape, and bears three strong setæ on the inner surface of either fork. Each seta is minutely ciliated, the first, however, bearing several much longer cilia about the middle of its length. There is only one external spine (the sixth of Mayer's nomenclature). The spine formula is therefore 5 + 5 instead of the normal 7 + 7.

Second Stage (fig. 3).—The following measurements are the average of the first ten specimens of a number measured, and the limits of variation in those specimens.

	Average length.		Minimum and maximum lengths.
1. Length of rostrum .	2.0 mm.	...	1.75 to 2.6 mm.
2. „ dorsal spine	2.7 „	...	2.5 „ 3.0 „
3. Tip to tip of spines .	5.7 „	...	5.1 „ 6.4 „
4. Length of body .	3.4 „	...	3.0 „ 4.4 „
5. „ thorax .	1.18 „	...	1.1 „ 1.25 „
6. Ratio of 4 to 3, 1 :	1.67.		

It will be seen that though the absolute length of the spines is much greater at this stage than in the preceding one, the ratio between the total length and the total length of the body has increased only by .01.

The more important differences between the zoæa at this period and that of the preceding one are the following:—In the second antenna the flagellum, which is barely indicated in the first stage, has grown out to nearly the length of the exopodite (spina mobilis). The exopodite and inner spinous prolongation of the stem are unchanged.

The maxillæ have changed but little in form, but bear more setæ. The scaphognathite of the second maxilla (fig. 10) has now nine setæ along its inner margin, and there are three terminal setæ instead of the single one of the preceding stage.

The first two maxillipedes are unchanged, except that the exopodite bears distally six ciliated setæ instead of four. The remaining thoracic legs are more distinctly developed, and there are traces of six gills on each side, i. e. those of the third maxillipedes and three succeeding limbs.

In the abdomen the pleopods are marked out as knobs on each segment except the first, which remains limbless throughout the larval development. The third, fourth, and fifth segments are produced into a short spinous process on each side. The sixth segment is separated from the telson. In the latter (fig. 4) the number of internal setæ is increased by one or even two pairs, so that there are either six or seven on each side altogether.

Third Stage.—Measurements (average and range of variation in eleven specimens) :

	Average length.	Minimum and maximum lengths.
1. Length of rostrum .	3·0 mm.	... 2·7 to 3·5 mm.
2. „ dorsal spine	3·6 „	... 3·4 „ 4·0 „
3. Tip to tip of spines .	8·6 „	... 8·0 „ 9·0 „
4. Length of body .	5·6 „	... 5·2 „ 7·0 „
5. „ thorax .	2·1 „	... 1·9 „ 2·7 „
6. Ratio of 4 to 3, 1 : 1·53.		
7. Length of third pleo- pod	·25 „	... ·2 „ ·35 „
8. Length of fourth abdo- minal segment . . .	·41 „	... ·3 „ ·45 „

At this stage the reduction in length of the dorsal and rostral spines as compared with the total length of the body has become very noticeable, and the increased completeness of the development of the limbs shows an evident approach towards the Megalopa.

The differences between the zoæa at this period and that of Stage 2 consists more in the increased development of parts already formed than in the acquisition of new ones. The internal branch of the first antenna is developing, and the base of the antenna shows a certain degree of swelling in preparation for the formation of the auditory organ. In the second antenna the increase in length of the flagellum is very striking, and it is now about twice the length of the exopodite, showing traces, beneath the cuticle, of segmentation. The mandibular palp is present as a small two-jointed process.

In the first maxilla no change has taken place, but in the second maxilla (fig. 11) the scaphognathite has not only increased in size, but is provided with a very greatly increased number of setæ, which fringe its edge, and are of more or less uniform size. Those of the posterior border are not longer or stouter than the rest. These changes are probably associated with the further development of the

gills, which perhaps become functional at this stage. The first and second maxillipedes retain their original form, but the exopodite bears now twelve setæ instead of six at its distal extremity. Each bears also at its base a small epipodite, but there is as yet no trace of gills. The succeeding six pairs of thoracic limbs are all well developed, and show distinct joints beneath the cuticle. The third maxillipede is the only one that bears an exopodite—a simple unjointed process,—the others developing directly to the adult form.

The gills of the posterior thoracic region (fig. 8) are all distinctly formed except that the podobranch of the third maxillipede is not yet separated from the epipodite. As yet also they have not acquired the lamellate form of the adult gill. In the abdomen the pleopods are further developed on the last five segments, each being about half the length of the segment succeeding it. The lateral spines of the segments are now more conspicuous than before.

The telson at this stage (fig. 5) has generally developed a new pair of setæ on its internal edge in front of the others, but in some specimens the number was found unequal on the two sides, the formula being normally 8 + 8, but occasionally 8 + 7.

Fourth Stage (fig. 7).—Measurements (average and variation in fourteen specimens):

	Average length.		Minimum and maximum length.
1. Length of rostrum .	3·4 mm. ...	3·1 to 3·8 mm.	
2. „ dorsal spine .	4·1 „ ...	3·8 „ 4·5 „	
3. Tip to tip of spine .	9·6 „ ...	8·95 „ 10·3 „	
4. Length of body .	7·3 „ ...	6·5 „ 7·8 „	
5. Length of thorax .	2·9 „ ...	2·7 „ 2·9 „	
6. Ratio of 4 to 3, 1 : 1·31.			
7. Length of third pleopod	·81 „ ...	·75 „ ·85 „	
8. „ fourth abdo- minal segment .	·63 „ ...	·6 „ ·65 „	

This stage is characterised by the greater development of the antennæ, gills, and pleopods, but otherwise shows no essential difference from the preceding one. In the first antenna the inner branch is more developed, and the outer branch shows signs of segmentation beneath the cuticle and an increased number of sensory rods. The base is much swollen, and the auditory pit is forming.

The second antenna has now a flagellum (endopodite) nearly two thirds the length of the rostrum, showing two distinct joints at its base and a number of indistinct joints beneath the cuticle. The exopodite and spinous process are relatively unimportant structures, and at the approach of the moult their contents are absorbed and only the chitinous cuticle remains.

The first maxilla shows no change, but in the second maxilla the setæ are more numerous upon the scaphognathite, though they are comparatively shorter than before.

The epipodites of the first and second maxillipedes are larger, but neither podobranchs nor arthrobranchs are formed.

In the third maxillipede, however, the podobranch is being separated from the epipodite, and the arthrobranchs are both present. The anterior arthrobranch, however, shows no signs of lamellar structure, though the posterior one, like the succeeding pairs of gills, is distinctly lamellate.

The gill formula at this stage is therefore as follows:

	A.	B.	C.	C'.	
VI.	Ep.	—	—	—	= Ep.
VII.	Ep.	—	—	—	= Ep.
VIII.	Ep.+1	1	1	—	= 3+Ep.
IX.	—	1	1	—	= 2
X.	—	—	—	1	= 1
XI.	—	—	—	1	= 1
XII.	—	—	—	—	
XIII.	—	—	—	—	
					<hr/> 7+3 Ep.

The pleopods are now well developed, each exceeding the length of the next succeeding segment. The first four pairs consist of a broad basal part bearing a long exopodite and a short stump representing the endopodite, but there are no setæ and no trace of segmentation. The fifth pair, on the sixth segment, are simple unbranched appendages. The telson is exactly the same as in the preceding stage.

The *Megalopa* (fig. 13).—Measurements (average of ten specimens):

Length of carapace	3·6 mm.
Breadth across third lateral spine	3·1 „
Length of antennæ	4·5 „

The last larval stage passes by a single moult to the *Megalopa*, which is distinctly recognisable as *Corystes*, though retaining certain features characteristic of the *zoæa*.

The rostrum and dorsal spine are still present, though very greatly reduced.

The rostrum has now the form of a broad plate extending forwards between the eyes, its lateral margin arched upwards and crenulated. Its extremity is trifid, the median process representing the last trace of the original long rostral spine and retaining the orange chromatophores of the previous stage, the lateral processes by which it is flanked being new formations. A few hairs are borne upon the upper anterior surface of the rostrum.

The dorsal spine is now an inconspicuous orange-red process, situated not immediately over, but somewhat behind the heart. From it a ridge runs forwards for some distance along the middle line of the carapace.

On either side of the middle line, in the region of the stomach, there is a single short spine on the dorsal surface. These spines appear first at this stage, and are lost again with the next moult.

Laterally the carapace bears three strong teeth on either side, the first immediately behind the eye, and the third above the first ambulatory leg. The postero-lateral margin

of the carapace is fringed with a number of setæ. The appendages have now taken on essentially the form of those of the adult. The second antennæ are considerably longer than the carapace, many-jointed, and provided with the characteristic dorsal and ventral row of setæ. The antennæ have, as already described, the same function of serving as a respiratory tube as they have in the adult. The mandible palp is now three-jointed, the distal joint bearing a number of setæ and overhanging the mouth opening in front.

The first maxilla differs from that of the preceding stage in the form of the endopodite, which is now not jointed, and bears but a single well-developed seta. This reduction in the number of setæ is remarkable from the fact that in the adult there is a rich clothing of setæ.

There is but little change in the form of the inner lobes, and the only change from this stage to the condition in the adult consists in a relative reduction of the superior lobe and an increase in number of spines.

In the second maxilla there is a great increase in size of the scaphognathite and simplification of the structure of the endopodite (fig. 12).

The first and second maxillipedes show an intermediate condition between the swimming limb of the zoæa and the masticatory limb of the adult. The two-jointed exopodite is practically unchanged, except that in the first pair it bears but five terminal setæ, and in the second pair eight. The endopodite of the first pair (fig. 15) is no longer jointed, but has not acquired the lamellate form characteristic of *Corystes*. The two basal joints are richly setiferous at their inner margin, and the epipodite is greatly developed. The endopodite of the second maxillipede (fig. 17) has practically the adult form, while the podobranch and small arthrobranch are both developed.

The third maxillipede develops directly to the adult form, the second joint of the endopodite having the characteristic anterior prolongation. The remaining thoracic legs have in all essential respects the form of those of the adult. The

abdomen still retains some larval characters. The lateral spines of segments 2—5 are still retained, and the telson still shows traces of bifurcation, being deeply indented posteriorly. The five pairs of pleopods have the shape characteristic of the typical Brachyurous Megalopa. Those of the first four pairs each consist of a stem bearing a long exopodite armed with numerous long ciliated setæ. The endopodite is very small, and interlocks with that of the opposite appendage as a retinaculum. The last (fifth) pair of pleopods have no endopodites, and are shorter than the telson itself.

First Post-larval Stage (fig. 14).—Measurement:

Length of carapace . . .	4.0 mm.
Breadth (across third lateral spines) . . .	3.2 „
Length of antennæ . . .	6.7 „

The cast skin of the specimen from which these measurements were taken had the following dimensions:

Length of carapace . . .	3.4 mm.
Breadth . . .	2.5 „
Length of antennæ . . .	4.0 „

The Megalopa stage lasts, according to my observations, from eighteen to twenty days, but possibly a more abundant food supply in natural conditions would somewhat shorten the period.

The young *Corystes* has now attained the structure of the adult in almost all respects. The rostral spine is reduced to an insignificant tubercle lying at the base of the indentation between the two anterior spines. The dorsal spine is completely lost, though a small orange chromatophore still marks its position on the carapace. The dorsal surface of the carapace is smooth, the median ridge of the previous stage and the two anterior dorsal spines having disappeared.

Besides the three lateral teeth of the Megalopa a fourth tooth is developed behind on each side close to the posterior edge of the carapace, so that the number characteristic of the adult is attained. The cephalo-thoracic appendages show no changes worth noting, except that the endopodite of the

first maxillipede has attained its final lamellar form (fig. 16). The abdomen, however, has changed considerably. It is now kept normally bent up under the body, the young crab having taken definitely to a burrowing habit. The first two segments are broad and flattened at the sides, while the remaining segments narrow out posteriorly and bear no lateral spines. All the segments bear setæ on their lateral margins. The telson has now an evenly rounded posterior margin.

The pleopods are no longer swimming organs, having lost all their setæ. The first four pairs remain biramous, and of about the same size as before, but the fifth pair is reduced to a simple stump. There is still no appendage upon the first abdominal segment, so that apparently in the female this appendage never develops, while in the male it is retarded till at least the second post-larval stage. I have hitherto obtained no later stage than that now under consideration, so that I cannot say at what period the distinctive sexual characters appear. The specimens of the first post-larval stage in my possession show also no difference in the relative size of the chelipeds.

CONCLUSION.

The *Corystidæ*, though placed by Milne Edwards (1834) and by Heller (1863) among the *Oxystomata*, have by more recent authors, such as Claus and Miers (1886), been assigned to the *Cyclometopa*. The resemblance between the *Corystidæ* and the true *Oxystomata* has been shown by Mr. Garstang (1897, etc.) to be largely superficial, and due to adaptive modifications of an essentially different character, though directed to the same ends. He has, in fact, brought forward clear evidence that the *Corystidæ* and the *Oxystomata* have been independently derived from *Cyclometopous* ancestors.

This view is to some extent supported by my observations on the development of *Corystes*, though the great uniformity in the structure of the *zoæa* throughout the *Brachyura* prevents any conclusion being drawn from the earlier stages.

In fact, it must be confessed that the most striking feature of the zœa of *Corystes*, namely, the great length of the spine, recalls the zœas of such *Oxystomata* as *Dorippe* and *Ethusa*—forms from which it differs essentially in other respects—more than those of the *Portunidæ*.

Still the final stages of the metamorphosis show that the peculiar emarginate rostrum of the adult (which recalls that of the *Oxystomata*) is preceded by a three-toothed rostral prominence which exactly resembles that found in most *Portunids*. That the central tooth represents more than a mere entogenetic stage in the reduction of the long rostral spine of the larva is also confirmed by the retention of a trifold rostrum in the adult of *Pseudocorystes* and *Trachycarcinus* (Faxon).

The existence of this *Portunid* stage in the development of *Corystes* was, I understand, the subject of a verbal communication made by Mr. Garstang to the Toronto meeting of the British Association in 1897 under the title "On Recapitulation in Development, as illustrated in the Life-history of the Masked Crab (*Corystes*).” As Mr. Garstang has been unable hitherto to write up his observations for publication, and as he informs me that the material at my disposal is more complete than in his own case, I am glad to be able to give a full account of the metamorphosis, and to confirm his observations. I may here express my indebtedness to him for his kind advice and many suggestions during the carrying out of my work.

PLYMOUTH; *May*, 1902.

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EXPLANATION OF PLATES 29—31,

Illustrating Mr. Robert Gurney's paper on “The Metamorphosis of *Corystes Cassivelaunus* (Pennant).”

All figures drawn with the aid of the camera lucida.

FIG. 1.—(× 32.) Zoæa of the first stage, showing distribution of chromatophores.

FIG. 2.—(× 100.) Telson of the first zoæa.

FIG. 3.—(× 47·5.) Zoæa of the second stage.

FIG. 4.—(× 100.) Telson of the second zoæa.

FIG. 5.—(× 47·5.) Telson of the third zoæa.

FIG. 6.—(× 35.) Third maxillipede of the first post-larval stage.

FIG. 7.—(× 26.) Zoæa of the fourth stage.

FIG. 8.—(× 65.) Second maxilla and thoracic appendages of the third zoæa.

Ep. 1—Ep. 3. Epipodites of maxillipedes 1—3.

a 1—a 3. Arthrobranchs 1—3.

p¹, p². First and second pleurobranchs.

mxp³. Third maxillipede.

- FIG. 9.—($\times 260$.) Second maxilla of the first zoea.
FIG. 10.—($\times 170$.) Second maxilla of the second zoea.
FIG. 11.—($\times 105$.) Second maxilla of the third zoea.
FIG. 12.—($\times 65$.) Second maxilla of the Megalopa.
FIG. 13.—($\times 20$.) The Megalopa.
FIG. 14.—($\times 20$.) The first post-larval stage.
FIG. 15.—($\times 45$.) First maxillipede of the Megalopa.
FIG. 16.—($\times 40$.) First maxillipede of the first post-larval stage.
FIG. 17.—($\times 45$.) Second maxillipede of the Megalopa.

Artificial Parthenogenesis and Fertilisation : A Review.

By

Thomas H. Bryce.

THIS article is an effort to gather together, in so far as they relate to the phenomena of fertilisation in the sea-urchin egg, the results obtained by experiments. It does not pretend to consider the problem of fertilisation as a whole, nor the phenomena save in Echinoderms, and no attempt will be made to establish comparisons with other forms in which the details may to some extent differ. The limitation to one form is in so far appropriate, that practically all the experiments have been made on Echinoderm eggs.

I have personally studied fertilisation in the egg of *Echinus esculentus*—specially in sections,—and though I have nothing fresh to add to the description of the facts, this article may in a measure be considered a sequel to a paper on maturation in the same form. In that paper my attention was chiefly directed to the chromosomes, and I did not follow out the results of observers in the experimental field, but as some of the phenomena described are of interest in connection with these results, I shall take the opportunity of returning to them.

The two mitotic divisions characteristic of the maturation phases, differ markedly from those which take place in the segmentation phases. In many respects there is a close resemblance to phenomena observed in the eggs of *Toxopneustes*, which develop parthenogenetically under the influence of magnesium chloride solution (Wilson, 1901).

On the dissolution of the nuclear membrane the site of the germinal vesicle is occupied by a "kinoplasmic" mass, derived either entirely from the nuclear network, or also partly from protoplasm differentiated on the distribution of the nuclear substance into it. In fixed material this area has a fibrillar appearance. This may be the result of the fixing reagents used, but in any case it indicates the accumulation at this part of protoplasm which has undergone some change in constitution physical or chemical. In the nuclear area, out of this material, asters are formed, and ultimately the first polar amphiaster. Besides the asters concerned in the formation of the bipolar figure, there are secondary asters, which seem to have only a temporary existence. In some few cases multipolar figures were observed. No structure recognisable as a centrosome or centriole was found before the germinal vesicle broke down, and therefore the centrosome was either derived from the nucleus, or formed *de novo* in the nuclear area. The astral radiations are confined to a small and superficial part of the egg, and a very unequal division results in the formation of the polar bodies. When the two divisions are over all the radiations and the remains of the kinoplasmic area disappear, the cytoplasm assumes its alveolar structure throughout, the nucleus retires from the surface, and no centrosome can be recognised in relation to it.

On the breaking down of the germinal vesicle the greater part of the nuclear material disappears as such, and not only is a change in the constitution and distribution of the protoplasm to be recognised, but experiment proves that the egg has undergone a physiological change of state. Whereas a spermatozoon can neither fertilise an egg with the germinal vesicle intact, nor a fragment without the nucleus (Delage, 1901), after the polar bodies are formed the egg becomes capable of fertilisation.¹ This cytoplasmic maturation, dependent, probably (Delage, 1901), on the influence of the

¹ For evidence and theories regarding the influence of the stage of maturation in Amphibian eggs see Bataillon (1901).

nuclear sap set free from the germinal vesicle, is accompanied by the conversion of the large vesicular nucleus related to the metabolic changes underlying the growth of the ovum, into a small morphologically equivalent nucleus, possessing the same number of chromosomes as the sperm nucleus. This has been proved by their enumeration when the nuclei undergo independent transformation, and the number is one half that found in the segmentation divisions.

The eggs thus matured remain, in the case of the sea-urchin, for a considerable time quiescent within the ovary before they are discharged—for the process of ripening in the ovary is a gradual one. When discharged into sea water it seems that, like the eggs of some other forms (O. Hertwig, 1893, p. 239), after lying for many hours unchanged, the sea-urchin eggs show spontaneously, karyokinetic transformation; for instance, R. Hertwig (1896) observed in eggs which had been deposited prematurely during transport, analogous changes to those produced by treatment with strychnine. This phenomenon is one apparently of wide range. In an interesting review entitled "Giebt es bei Wirbeltieren Parthenogenesis" (1900), Bounet, after examination of all the literature up to that date, comes to the conclusion that, according to our present knowledge, the phenomena in vertebrates are due to degenerative divisions, and in meroblastic eggs to fragmentations, and the alleged parthenogenetically divided tubal, uterine or laid eggs, are either over-ripe, and therefore badly fertilised, or are eggs normally fertilised with defective spermatozoa. In the light of the facts of artificial parthenogenesis, it may be that this segmentation in unfertilised eggs, at least in certain invertebrates, is an effort in the direction of true parthenogenesis which is abortive, the egg dying before the tardy process is accomplished.

In 1876 Greeff described parthenogenetic development in *Asterocanthion*. The eggs were obtained from animals early in the season, before the spermatozoa were mobile, and the blastulæ formed differed from those produced in normal fertilisation. O. Hertwig (1890) recorded some observations

on spontaneous parthenogenesis. In confirmation of Fol, he found that eggs from fully-matured animals did not segment spontaneously, but only after a considerable time underwent changes considered pathological. The nucleus enlarged more and more, and after ten to fifteen hours the eggs died and fragmented. Only among hundreds of eggs here and there one had divided into two. At Trieste, however, in a season when the animals were late in maturing, and at a time when males were rarely got, he observed in a limited number of cases (in *Asterias glacialis*, and *Asteropecten*) that after the polar mitosis had occurred, the nucleus did not come to rest, but continued to divide. There resulted an irregular division, but here and there a blastula was found which had no vitelline membrane. Into the interesting observations and suggestions regarding the failure of the second polar body extrusion, and the union of two vesicular nuclei in the egg, we cannot here enter. The main point established was, that fully-matured eggs did not develop parthenogenetically, but that in some few cases immature eggs did divide irregularly, and in a small number of cases blastulæ were formed. A number of observers have described the occurrence of natural parthenogenesis in Echinoderms, and it is an open question; but apart from its possible relation to immaturity of the ovum, the sources of error in the matter of infection by spermatozoa are so many, and the causes which artificially start parthenogenetic development in certain cases are so slight, that all cases of so-called "natural parthenogenesis" are open to suspicion, but even granting that it may occur, it is a matter of no great moment in the question of "artificial parthenogenesis." It would be only additional evidence of the fact, that there is in these forms a tendency to parthenogenetic development, which, however, does not normally occur.

Mitotic division may be excited in unfertilised eggs in a variety of ways.

First, by increasing the degree of concentration of the sea water (Morgan, Hunter), or by increasing the osmotic

pressure in various other ways (Loeb). This may be done by adding various inorganic salts to the sea water, especially the salts of magnesium, potassium, sodium, and calcium, in definite proportions (Loeb, Morgan, and others), or by adding sugar or urea (Loeb). Other salts have also given results, e.g. chloride of manganese (Delage). The effect is produced when the eggs, after being left from half an hour to two hours in the solution, are transferred to pure sea water, and is due to the disturbance of the osmotic pressure leading to loss of water by the egg, followed by rehydration (Bataillon, Loeb, Giard, etc.), not to specific chemical stimulation.

The nuclear activity may also be roused by other chemical bodies, as strychnine (Hertwigs, Morgan), chloroform, ether, alcohol, by lack of oxygen (Mathews), by very dilute hydrochloric acid (Loeb, Delage).

Further, purely physical agents may have the same effect—heat (Mathews, Bataillon, Delage, Viguier), cold (Morgan, Greeley), and, most important, agitation (Mathews). Mathews had previously proved for *Asterias*, and Morgan for *Arbacia* also, that shaking of unripe eggs caused them to form the polar bodies—the shaking presumably causing dissolution of the nuclear membrane. Ripe eggs of *Asterias*, but not of sea-urchin, act in the same way, but only after they have lain some time in water; after two hours larvæ begin to appear on shaking; after four hours, hard shaking produces a large proportion of larvæ, and the mere transference of the eggs by a pipette from one vessel to another is sufficient to form a few larvæ. A few hours later the slight amount of shock experienced in the transference of the eggs, causes a large number to begin to develop, though they do not go beyond the late segmentation stages. At this time shaking causes all to develop, but none reach the blastula stage. Loeb and Fischer have extended this observation to the Annelids, *Chætopterus* and *Amphitrite*.

The mitotic phenomena produced artificially are apt to be irregular, and the division of the cell body is often unequal

when it occurs. Thus the nucleus may divide repeatedly without division of the cytoplasm, and then the egg may break into as many segments as there are nuclei (Wilson and others). It is only in a relatively small proportion of eggs that division is regular enough to permit of development to the larval stage. Further, the eggs of the same species behave capriciously to the same agents under different conditions (temperature, etc.), and the eggs of closely allied species seem to react differently to the same agent.

There is no reasonable doubt, however, that true artificial parthenogenetic development has been demonstrated for the Echinoderms—sea-urchins and star-fish—and for at least two Annelids, though the same amount of independent testimony is not available for the latter.

Actual development to a larval stage has been obtained only by certain of the agents enumerated above.

1. Increase of Osmotic Pressure.—The most successful results (Loeb, 1902) are obtained at a temperature about 20° C., by the addition of the chlorides of potassium or sodium to sea water, the optimum degree of concentration being determined by experiment for each set of observations.¹ After the eggs have remained in this for half an hour to two hours, the optimum being again tested by experiment, they are restored to normal sea water.

Sea-urchins (Loeb, Wilson, Giard, Prowazek, Delage, Viguier, Hunter). Annelids: Chætopterus, Amphitrite, Nereis (Loeb, Fischer).

2. Agitation. — Asterias (Mathews), Chætopterus and Amphitrite (Loeb, Fischer), but not sea-urchins (Mathews, Viguier).

3. Elevation of Temperature.—Asterias during maturation (Delage) ; not for ripe eggs (Greeley, Viguier).

4. Depression of Temperature.—Asterias (Greeley) ; not sea-urchins (Viguier).

¹ Loeb (1902) uses a stock solution of $2\frac{1}{2}$ n. HCl, and adds this in different proportions, 8, 10, 12, 14, 16, 18 c.cm., to 100 c.c. of sea water in six vessels to determine the best grade of concentration.

5. Exposure to weak HCl in sea water, and subsequent restoration to pure sea water. *Asterias* (Loeb, Delage).

6. Continuous exposure to a solution of a specific chemical substance at the same osmotic pressure as normal sea water.

Potassium Chloride. *Chaetopterus* (Loeb).

Calcium Chloride. *Amphitrite* (Fischer).

The Ions of potassium and calcium are said to be specific for these forms respectively.¹

With regard to the influence of the state of maturation, Delage gives results to show that in *Asterias glacialis*, when the eggs are placed in sea water to which is added an equal quantity of a solution of HCl, raising the molecular concentrations of the mixture to 0.660, different results are got according to the stage of maturation. Among the eggs placed in the liquid before maturation, 20 per cent. of blastulæ were got, at the appearance of the first polar body 95 per cent., and after the appearance of the second polar body 5 per cent., while none of the controls showed any normal segmentation.

From all this it seems that changes in the osmotic pressure between the egg and its surrounding medium, and mechanical agitation, are the chief agents so far as yet

¹ Delage ('Compt. Rendus de l'Acad. des Sciences,' October 13th and 20th, 1902) announces that he has found an agent which is as certain and effective as the spermatozoon, in producing development to advanced larval stages, in *Asterias*. It is sea water aerated by carbonic acid gas, and at the same osmotic pressure as ordinary sea water (or lower?). When the eggs, at what he calls the "critical stage"—i.e. when the nuclear membrane of the germinal vesicle is dissolved, up to the expulsion of the first polar body—are placed in this, and after one hour transferred to pure sea water, practically all the eggs develop. His view is, that the maturation is arrested temporarily, and on restoration to pure sea water, the carbonic acid gas is quickly eliminated and division proceeds; but it is not partial, as in the polar mitoses, but complete, and goes on to the formation of the normal larval forms. The result is not obtained at a stage after the polar bodies are extruded and the ovum has again come to rest, nor is it applicable in sea-urchin, in which the maturation is over before the ova are shed. His theory as to the action of the gas is, that it is a temporary poison which arrests maturation completely, and is quickly removed afterwards without altering the characters of the protoplasm.

known, which tend to the production of artificial parthenogenesis, but that in the case of the Annelids there is evidence to show that certain Ions may have a specific effect.

According to Loeb (1902) the solutions must act, first, by favouring the solution or dissolution of the nuclear membrane; and second, by changing, in some sense, the physical properties of the protoplasm (viscosity, etc.).

Mathews (1900), as a conclusion from his experiments on *Arbacia* eggs, pointed out that the known methods of causing liquefaction in protoplasm will induce karyokinesis in these eggs, and also shows that loss of water has a liquefying action.

Before considering further the bearing of the physiological and physico-chemical conceptions regarding fertilisation, I shall proceed to the morphological changes which have been described in unfertilised eggs which undergo parthenogenetic development.

R. Hertwig (1896) studied the changes in the egg after treatment with strychnine. On the breaking down of the nucleus, half spindles, and in a few cases whole spindles, supposed to arise from the fan spindles, were formed. The fan spindle fibres he regarded as derived from the achromatic network of the nucleus. The chromosomes derived from the nucleoli became attached to the primary rays. Later, protoplasmic rays also appeared, centering on the focal point of the half spindle. At this central point, and derived from the central parts of the rays, there appeared a rounded body resembling in every way a centrosome, though none such was to be found before the nucleus broke down. The body was an ovocentrum, formed from the achromatic portion of the nucleus, and, according to Hertwig, the individualised centrosome is ultimately a derivative of the nucleus—is, in fact, an achromatic nucleus.

Doflein (1897), contrariwise, examined the phenomena of karyokinesis of the sperm nucleus in eggs which, after fertilisation, had been treated by chloral solution after the manner of the experiment of O. and R. Hertwig. The nuclei

did not unite, but underwent independent transformation. Doflein, like R. Hertwig, considered the middle piece of the spermatozoon as equivalent to the centrosome, and from the experiments concluded, that from the centrosome a complete spindle could form, and out of this, again, the achromatic nuclear network. Thus, compared with Hertwig's results, the ripe sperm nucleus contains all the parts, even as the ripe egg nucleus, which are necessary for a further development.

In Hertwig's results we have evidence of a centrosome arising from the nucleus *de novo*. Morgan, in 1896, described the formation of artificial astrospheres in the cytoplasm of the eggs of *Arbacia* treated by salt solutions, and from his further observations published in 1899 and 1900 he decided, that in spite of certain differences these artificial astrospheres corresponded to the normal spheres which occur at the apices of the spindles in the segmentation stages; further (1900), that both artificial and normal spheres are due to accumulation of a specific substance, and that the yolk spheres are excluded from the substance of the astrospheres.

His view of the astral radiations is that they serve to transport the chromosomes, but are not concerned in the division of the cytoplasm.

Evidence of free formation of the centrosomes is found also in the appearance of asters in the cytoplasm in various forms, *Echinus* among them, on the breaking down of the germinal vesicle in maturation.

Boveri (1901) in essence accepted Hertwig's definition of the structure described by him as an ovocentrum, and its origin apparently *de novo*. He argued that phylogenetically the centrosome is an individualised cytocentrum, derived from a centro-nucleus in which the centrosome or its equivalent is not differentiated from the chromatin nucleus. To the nucleus of the sea-urchin egg must necessarily be attributed the properties of a centro-nucleus, with the capacity of producing out of itself, under the action of certain stimuli, individualised centrosomes, when such fail to be supplied in the normal way in fertilisation. If even under

similar conditions, the sperm centrosome be present, the cyto-centrum remains latent. The centrosome looked at in this way, is not a specific cell organ in the sense that it must consist of a specific chemical substance, but that parts of a substance contained in the nucleus, undergoing certain changes, and grouping themselves together, are organised into a centrosome.

Thus the ovocentrum of the sea-urchin egg is not to be considered an individualised centrosome, but an intranuclear latent cytozentrum, and the nucleus is a centronucleus. Thus the centrosome in such a case is not something strictly new, but arises by the transformation in a definite manner of a cytozentrum already present. It is a case not of new formation, but of "reparation." "Gervisse Centronuclei sind im stande unter bestimmten Bedingungen Centrosomen zu reparieren."

Morgan's artificial astrospheres he did not admit to have true centrosomes—the essential character of capacity for division was not proved for them.

This brings me to Wilson's very interesting and important paper on the morphological phenomena in parthenogenetic eggs.

The main results are that under the influence of the magnesium chloride solution, not only are asters produced *de novo* in connection with the nucleus, but also in the cytoplasm. "Not only the asters connected with chromosomes (nuclear asters), but also the supernumerary asters unconnected with nuclear matter (cytasters), may multiply by division; the cytasters contain deeply staining central granules indistinguishable from centrosomes, that divide to form the centres of the daughter asters. These asters operate with greater or less energy as centres of cytoplasmic division. Typical cytasters, often containing deeply staining central granules resembling centrosomes, are formed in the magnesium solution in enucleated egg fragments produced by shaking the unfertilised eggs to pieces, and these asters likewise may multiply by division, though

no cytoplasmic cleavage takes place. The cleavage centrosomes first make their appearance outside the nucleus, but directly on the nuclear membrane, and the evidence renders it nearly certain that they arise by the division of a single primary egg centrosome that is formed *de novo*. All the evidence goes to show that the cleavage centrosomes are of the same general nature as the central bodies of the cytasters."

Among many interesting details I will refer here only to the changes described for eggs which underwent segmentation, and were capable of developing into swimming embryos, because in certain particulars they are reminiscent of what takes place in the formation of the first polar amphiaster.

I may summarise as follows:—(1) The first change that occurs is a coarsening in the appearance of the protoplasm, better marked in eggs treated by stronger solutions. (2) A primary radiation appears centering on the nucleus, better marked in eggs treated with weaker solutions. (3) A varying number of secondary radiations appear in eggs especially treated with stronger solutions. The extent of the primary radiations is inversely in proportion to the number of the secondary radiations. These latter appear as vague clear spots in the cytoplasm, which gradually become surrounded with radiations, and finally assume the form of asters. They always appear *in situ*, and do not change their position till a later period. (4) Coincident with the appearance of the radiations there is a gradual growth of the nucleus. (5) Round the nucleus appears a clear perinuclear zone of hyaloplasm. (6) The nuclear membrane fades out, and a vague irregular clear space is left, to which the hyaline zone contributes. (7) The rays then diminish, and, indeed, almost disappear.

The eggs at this point were restored to pure sea water, and after a pause the radiations reappear and advance centrifugally towards the periphery. In eggs capable of development the principal rays are now focussed on two centres at opposite poles of the nuclear area, which now forms a spindle connecting the two asters. If the amphiaster is typical,

division proceeds as in normal fertilisation. If more than two asters are formed from the nuclear area, multipolar figures form, and irregular cleavage results. If there is only a single radiation which does not resolve itself into a bipolar figure, the egg never properly segments, but there are regularly alternating phases of nuclear transformation.

Analysing the meaning of the phenomena, Wilson says, "We may therefore state that the first general effect of the stimulus, whether the magnesium solution or the spermatozoon, is to arouse an activity of the cytoplasm, one result of which is the establishment of a centripetal movement of the hyaloplasm towards one or more points at which the hyaloplasm accumulates." The rays in this view are the expression, in part at any rate, of centripetal currents, and the substance flowing in, is the hyaloplasm or interalveolar substance. The hyaloplasm spheres at the centres of the asters are local accumulations of this hyaloplasm. In fixed material, studied in sections, the radiations are fibrillar in appearance, and as they stain much more deeply than the general network the hyaloplasm in the rays must probably have undergone some physical or chemical change. The centrosome is a well-defined body of considerable size and of spongy consistence, composed of intensely staining granules, which often give the centrosome the appearance of a minute nucleus containing a chromatin reticulum. The hyaloplasm spheres in the living egg correspond to the centrosome, the clear area round it, and the innermost darkly staining radiated zone of the aster taken together.

Thus Wilson has proved that structures which cannot be distinguished morphologically from "true centrosomes" appear in the cytoplasm *de novo*; and further, that they divide to form the apices of bipolar figures, even in enucleated fragments.

In a recent paper Meves (1902), using Boveri's nomenclature, expresses the view that the centrosome is only the mantle of the centriole, and is only present in rapidly-dividing cells like the blastomeres. The "Doppelkörperchen" of the

tissue-cells are to be considered as centrioles, and “*nur von den Centriolen nicht aber von den Centrosomen, kann daher gelten, dass sie allgemeine und dauernde Zellorgane sind.*” The results of Morgan and Wilson can only then be held to prove that centrioles under certain conditions may, by the action of salt solutions, be excited to form centrosomes and radiations round them, for their results might be explained by a multiplication of the two centrioles which the egg has derived from the last division of the division period, and the distribution of these centrioles through the cell. Even in enucleated fragments there is no proof that the fragment did not contain the centriole of the cell.

Such a supposition admits of neither proof nor disproof, and the presence of a free “centriole” in the unfertilised sea-urchin egg has not been demonstrated. I have seen in young oocytes minute bodies, stained black with iron hæmatoxylin—sometimes double bodies,—but I have not been able to convince myself that they are more than accidents of staining and fixing.

Turning now to the phenomena of fertilisation in the sea-urchin, there is to be recognised (1) a local stimulation at the place of contact of the chosen spermatozoon,¹ manifested by the streaming out of the protoplasm to form the entrance cone. (2) A general stimulation, manifested by the throwing off of the vitelline membrane, and by a change in the constitution of the protoplasm. It becomes more viscid for a time (Morgan); a funnel-shaped area of darkly staining substance follows the path of the sperm head (Wilson). (3) A protoplasmic movement focussed on the situation of the middle piece giving rise to the sperm aster. This appears soon after the entrance of the spermatozoon, when the head has begun a movement of rotation. The rotation goes on

Buller (1902) has studied the question of the bearing of chemotaxis on fertilisation in Echinoderms. His conclusion is that chemotaxis plays no rôle in bringing the sexual elements together. The meeting is a matter of chance. The passage through the gelatinous coat is radial in direction, and probably purely mechanical, though possibly due to stereotaxis.

through 180° till the base of the conical sperm head is directed inwards. The rays of the aster now extend widely, and at their centre is a clear area. Meantime the sperm head becomes converted into a small round nucleus. The movement of the sperm head is, at first, radial; then there is a change, and it assumes a new direction towards a point not quite in the centre of the egg; when this change of path is taken up the egg nucleus begins to move towards the point where the nuclei ultimately meet (Wilson and Giardina). The aster now comes in contact with the egg nucleus, and as the nuclei approach, the clear area at its centre spreads out over its side. The aster then divides and the nuclei conjugate. The radiations now die down during a pause in which the nucleus grows in size (Wilson), to redevelop again focussed at the poles of the nucleus.

According to Hertwig, Doflein, Erlanger, and Wilson's earlier account, the centrosome corresponds to the whole middle piece, but later Wilson described the middle piece as cast aside, and in the centre of the aster is a small darkly-staining granule. Boveri (1901) represents the sperm centrosome as a spherical body smaller than the middle piece, and containing within it two centrioles shortly after its entrance into the egg.

Various other observers have represented a dark-staining granule at the centre of the aster. My own observations are inconclusive, and do not warrant me in expressing an opinion.¹

¹ The character of the fully-formed centrosome in the sea-urchin egg is still subject to difference of opinion. The form in which I see it in osmic acid material is that of a largish sphere of very finely alveolar structure. In Wilson's papers on magnesium and etherised eggs, "it appears as a well-defined body of considerable size, consisting of intensely stained granules, which often give the centrosome exactly the appearance of a minute nucleus containing a chromatin network." This becomes in the anaphases more homogeneous, and flattens down into a plate-form, which in the telophases often lies directly on the membrane of the newly-formed nucleus precisely as Boveri (1901) has described for *Echinus*. Boveri (1901) represents it in several forms. In one set of preparations it is a largish sphere of very finely alveolar

The essential difference between the processes seen in magnesium eggs and normal fertilisation is that whereas in fertilisation there is only one, and that a definitely localised point of astral activity, in the magnesium eggs there are a number of foci, and development in large measure depends on the accident of their number in the nuclear area.

There is the same want of unity of purpose that is seen in polyspermic eggs, in which the number of points of astral activity depends on the number of spermatozoa which gain an entrance.

It has long been recognised that the union of the nuclei and the initiation of division are co-ordinated, but in a measure independent factors in fertilisation. Parthenogenetic development under artificial agents is the latest proof of this. The possibility of the development of enucleated egg fragments when entered by a spermatozoon, as described by Boveri, and afterwards named merogony by Delage, is another. Either nucleus is sufficient in itself.

With the problems underlying the nuclear conjugation this article is not concerned. It starts from the assumption that the union of equivalent nuclei is the end of fertilisation, but not the means (Boveri).

The cause of the nuclear conjugation is not as yet understood. The first possibility is that the aster is concerned in bringing them together. Giardina (October, 1902) brings the latest suggestion on this line. Starting from the basis of the alveolar structure of protoplasm, he suggests that the aster is the expression of both centripetal and centrifugal currents. The centrosome is concerned in the diffusion of chemotrophic substances into the egg, while at the same time structure. In another set, in which the centrosome had reacted differently, there is a centriole within the centrosome, which divides before the centrosome, so that it is double in the metaphase. In Wilson's earlier account there was no central body, but in later descriptions there was a mass of granules in a well-defined sphere, which succeeded a single granule of earlier stages. In my previous paper, I regret that I misrepresented Professor Wilson's nomenclature by referring to this as his centrosome. The sphere, as a whole, is named the centrosome. See note to page 314, "The cell, etc.," 1900.

the hyaloplasm flows in towards the centre. He points out that the germ nucleus does not move till the rays of the aster have reached it, and the aster has assumed a position of equilibrium towards the centre of the egg. The union is thus the result of the chemotactic forces of which the aster is the expression.

Wilson (1901 B) shows, however, that the nuclei may unite in the entire absence of an aster. When eggs, immediately after fertilisation, are placed in a weak solution of chloral (O. and R. Hertwig), or ether (Wilson), no aster is developed, but when replaced in sea water the rays reappear and the nuclei unite. In a certain proportion of cases, which will be referred to later, the nuclei remain apart and undergo independent transformation; but in some instances, also while the eggs are still in ether, the nuclei enlarge, and later conjugate in the entire absence of an aster. This happens, however, only when the spermatozoon has entered at a point not too far from the egg nucleus. Giardina holds that this fact, and the other—that the nuclei quickly unite whenever the eggs are put in pure sea water, and the aster develops,—makes Wilson's observation insufficient to exclude his hypothesis. Other explanations, such as mass attraction and direct chemical attraction, both observers reject. Wilson thinks the latter improbable. Again, the idea of protoplasmic currents such as suggested by Butschli, Erlanger, and Conklin, is not proved by actual evidence in normal conditions in the sea-urchin egg (Wilson). The changes of shape of the germ nucleus might suggest amœboid movement on its part; but, again, this does not apply to the sperm nucleus, which travels through a longer path (Wilson). The changes in form might be due to the exercise of chemotactic forces on the nucleus (Giardina).

The phenomenon described by Boveri (1888) under the name of "Partial Fertilisation," has recently been worked out in detail in Boveri's fixed preparations by Teichmann (1902). The method by which the results were obtained was that eggs which had lain fourteen hours in unrenewed

sea water were fertilised with spermatozoa, which were treated with a .05 per cent. solution of potassium hydrate until only a few were mobile. While polyspermy occurred in more than half the eggs, the remainder were fertilised by a single spermatozoon. In these cases, however, the sperm nucleus did not unite with the germ nucleus, but the aster became detached from it, and advanced alone to the germ nucleus, a bipolar figure was formed and division proceeded. The sperm nucleus took no share in the process, but passed unaltered into one of the blastomeres. Later, however, either in the two- or the four-cell space, it broke up into its chromosomes, which entered into the equatorial plate of the cell in which it was included, and which now divided like its neighbours. Such eggs were capable of developing to the blastula stage.

The question presented itself: Was this aster and the amphiaser the result of the activity of an ovocentrum, or were they the product of the sperm aster?

In monospermic eggs Teichmann found the early stages very scarce, and, though very suggestive, too few for absolute proof, and the phenomena seen in dyspermic eggs are described to fill the gap. It may be admitted that in these eggs, in spite of the apparent inactivity of the sperm nucleus, the sperm aster with its centrum is the operative factor in starting the developmental process. The appearances are very similar to those in the etherised eggs described by Wilson (1901). In that form, as in *Echinus*, the nuclei conjugate when they are very unequal in size, and before the division of the aster. In *Asterias* and other forms an amphiaser is developed before conjugation, and the nuclei are nearly equal in size. In the experiments the union was delayed, as in "partial fertilisation," and the amphiaser was formed before the conjugation.

Among Teichmann's observations I shall refer only to those of monospermic eggs. The main feature is the detachment of the sperm aster from its nucleus, its application to the egg nucleus, and its normal division, followed by normal segmentation. The fate of the sperm nucleus depends on the

position it assumes in the egg, relative to the cleavage plane. If it lies outside the equatorial plate of the spindle it passes unchanged into one of the blastomeres; if it lies within the field of the first spindle, it does not actually unite with the chromatin of the female nucleus, but its chromatin undergoes a marked relaxation. Though it shows a marked resistance to the tractive forces, it is drawn out and torn into several shreds. It thus passes undivided into one of the blastomeres, and no chromatin elements derived from it are found at the poles of the spindle. In several cases where the nucleus lay exactly at the equator, and the traction of the poles was nearly equal, it was observed that the chromatin mass was much broken up, and was torn into two parts. The cleavage of the cell body may have helped to complete the division. The loosening of the sperm chromatin mass in the first spindle seems to have broken its power of resistance, for when the next division is initiated, the two nuclei lying side by side in one of the blastomeres unite in the equatorial plate stage, and the chromatin of both is equally distributed in the next division. The number of chromosomes is now different in the blastomeres, sometimes double, perhaps quadruple in some cases (though an accurate count was not possible), as if the chromosomes of the sperm nucleus emerged in double number, though the first division was suppressed.

The sperm nucleus in the case where it has not lain within the power of the spindle in the first division, may now, in analogous fashion, be caught in the second division, to unite later with the chromatin of one of the blastomeres of the four-cell stage, or it may even pass over into the eight-cell stage, as seen in living eggs by Boveri.

Teichmann concludes that the radiations are derived from the sperm centrosome as their starting point, and on the supposition that the centrosome is introduced by the spermatozoon into the egg, that it has suffered less from the chemical reagent than the nucleus. The centrosome behaves as in ordinary fertilisation, the sperm nucleus is passive, and

seems to form no hindrance to the normal processes in the egg, and it seems to be of no significance, whether it enters earlier or later into union with one of the descendants of the egg nucleus. The difference between the phenomena of "partial fertilisation" and the normal process is the non-union of the nuclei. In certain cases Boveri (1890) described an independent transformation of the nuclei under normal conditions, but the elements from both entered into the equatorial plate of the first cleavage spindle, and normal division took place. The mere want of union is not of moment, if the sperm nucleus lies near enough the germ nucleus to be influenced by the nuclear fluid of the egg nucleus. The absence of this hastening factor may explain the fact that the karyokinesis of the sperm nucleus in enucleated fragments is much slower than in the cleavage spindle. But such an explanation alone will not hold for cases of dyspermy in these experiments, where the sperm nuclear descendants remain far behind the derivatives of the egg nucleus, and it must be assumed that a change has taken place in the sperm nucleus itself, a kind of paresis, produced by the potassium hydrate. This holds for the monospermic eggs also, and explains why, even in spite of its position, the nucleus does not enter into union.

Another factor is a change in the egg, defined as an over-ripeness. In many cases the germ nucleus is a stage ahead, compared with the normal process, of the centrosome. When the centrosome met the egg nucleus, the latter must already have been in a way prepared for division, and this great readiness to enter into division may be part explanation of the lagging behind of the sperm nucleus. There has not been time for the sperm nucleus to undergo transformation before the egg nucleus has submitted to division. Teichmann does not explain in what the over-ripeness consists. It may perhaps be that, since the eggs had lain fourteen hours in unrenewed sea water, the early preparatory stages of the natural transformation had supervened, which takes place in eggs after lying long in sea water.

Regarding the main point, it may be admitted that the aster and its centrosome here concerned is that belonging to the sperm nucleus, and that in its behaviour we have a beautiful demonstration of the independence of the two factors in fertilisation, or, in other words, of the two functions of the spermatozoon, and that the two functions have been disturbed in unequal degree. At the same time the egg protoplasm, after the fourteen hours' sojourn in unrenewed sea water, was approaching to that stage in which it acquires spontaneously the tendency to develop astral activities, and it might be held that the conditions are the same as in magnesium eggs in which, as a result of a general stimulation, asters and centrosomes which are unconnected with the nucleus appear *de novo* in the cytoplasm. While the results are of interest in connection with the apparent independence of the factors in fertilisation, they also show how they are co-ordinated together. The sperm nucleus becomes dissociated from the aster, and fails of union, because it has not undergone the transformation which properly corresponds to the phase reached in the cycle of the centrosomal changes. Further, while the nuclei may be resolved into chromosomes before union, and yet unite in the equatorial plate stage, a certain stage in the transformation of the dense mass of chromatin of the sperm head into a nucleus with distinct chromatin network, must be reached before union can take place. This seems to show that several co-ordinated factors are at work in the nuclear conjugation.

Further insight into the behaviour of the factors in fertilisation is given by an experiment described by Ziegler (1898). This consisted in carrying newly-fertilised eggs by a gentle current of water in his compressorium against threads of cotton wool. The egg was caught on a thread and nearly cut through, leaving only a slender bridge of protoplasm between the two portions of the egg. The one contained the sperm nucleus, the other the germ nucleus. While the sperm nucleus regularly divided, followed by division of the cytoplasm, the egg nucleus merely underwent

alternate changes of disintegration and reconstruction without division of the cytoplasm. Radiations appeared and disappeared, and after three cycles, owing to the segmentation of the portions containing the sperm nucleus, the egg-nuclear portion became detached and disintegrated. In another experiment the egg-nuclear portion underwent changes of form suggesting abortive attempts at cleavage. The mitotic transformation of the egg nucleus was not synchronous with that of the sperm nucleus, but always a little behind.

These observations show that under the conditions of the experiments the egg nucleus is excited to division without direct contact with the sperm nucleus or aster, but that the mitotic phenomena are ineffective to produce cytoplasmic cleavage. Ziegler refers this to the general stimulation of the egg by the spermatozoon, manifested also by the throwing off of the vitelline membrane. Boveri has shown that the same phenomena occur in egg fragments produced by shaking some minutes after fertilisation, and he (1902) refers to cases of this kind in which he has observed divisions of the nucleus followed by cell cleavage. The division was repeated a second time, and thus the four-cell stage was reached, but development then ceased. Another example of the effect of this general stimulation is to be seen (Boveri, 1902) in the cases in which the egg is incited to throw off the polar bodies by the entrance of the spermatozoon.

This brings me to a further reference to Wilson's observations on etherised eggs (1901 B). As has already been said, under this treatment the sperm and germ nuclei remain apart and undergo independently karyokinetic transformation. "The most striking fact is that, while the sperm aster often gives rise to a perfect and symmetrical bipolar figure, the egg nucleus in a great number of cases produces a monaster, which seems at first incapable of resolving itself into a bipolar figure." In typical cases the egg nucleus gives rise to a monaster such as described by Hertwig ('96), and such as occurs in magnesium eggs. While the egg monaster does not at first give rise to a dicentric figure, it does so later, as

may be gathered from the description of an egg continuously observed in the living state. At the height of its development the egg monaster lay at one side, the sperm amphister at the other, and no spindle was formed between them. The egg divided into three cells, two larger and somewhat irregular containing two daughter sperm nuclei, and a small one in which the single egg nucleus re-formed. At the second division each of the sperm nuclei gave rise to a perfect amphister, and divided into two, the accompanying cytoplasmic division resulting in the formation of two complete cells and one binucleate cell. The single egg nucleus gave rise to a tetraster, and divided into three cells, one binucleate, the nuclei of the latter quickly fusing together. The embryo now consisted of six cells—three containing maternal, three paternal nuclei. At the ensuing division fifteen cells were formed, of which eight larger ones contained paternal nuclei, while seven much smaller ones containing maternal nuclei lay in a definite group at one side. The egg observed afterwards died. Wilson has not seen an egg monaster become dicentric at the first division, but the above observations prove that it may operate as an effective division centre, without establishing a spindle connection with either of the sperm asters, and that it may divide later. A centrosome was demonstrated in the monaster, in the same form as in the sperm aster, and as in magnesium eggs. The possible action of the chemical as the exciting agent of the karyokinetic transformation was excluded by control experiments, and it was therefore concluded, that it was due to a stimulus effected by the spermatozoon, as in Ziegler's experiment. These observations, added to the results obtained in the magnesium eggs, "demonstrate that under appropriate stimulus the egg may give rise to a centrosome capable of progressive division, but the etherised eggs show in the clearest manner that this centrosome is less effective than the sperm centrosome."

I shall not venture on the general problem of the asters and centrosomes. It will suffice for the present purpose if it be

admitted that, without prejudice to the question either of the individuality, or the persistence of the centrosome, the body and its aster represent a kinetic phase of protoplasm, which reveals itself in cycles of activity, and that the centrosomes and asters constitute together, in some sense, a divisional apparatus, though that term is not used in any definite mechanical sense.

The egg both before and after maturation lacks the power, for and by itself, to produce in normal circumstances such a divisional apparatus as will regularly and equally divide the cell.

In Ziegler's and Boveri's experiments on separated portions of the egg containing only the egg nucleus, a divisional apparatus is called up under the general stimulation of the spermatozoon; but it is ineffective, or only very partially effective. In the etherised eggs it is slow in appearing, and less effective than that associated with the sperm nucleus. In magnesium eggs the effect of the disturbance of equilibrium is to cause a change of state in the protoplasm which results in the differentiation at many foci of kinetic centres, and it is only in the cases where a single such centre, which divides into two, appears in the nuclear area, or at most two centres, that normal division proceeds.

In fertilisation there is only one kinetic centre, and this is localised on the middle piece of the spermatozoon. Its activities are rapidly unfolded, and dominate all the other latent astral activities of the egg. "The latent capacity of both nucleus and cytoplasm to give rise to centrosomes is in this case wholly inhibited."¹ By union of the nuclei its activity is transferred to the cleavage nucleus, and "becomes a part of an activity on the part of the egg nucleus that would have ensued even had the germ nuclei not united."¹

Thus it may be said that the spermatozoon supplies the lack in the egg, by providing a powerful and effective "divisional apparatus." How is this effected? Does the spermatozoon act by giving a general or diffused stimulus

¹ Wilson, 1901 A, pp. 581, 582.

to the egg, or by disturbing the general equilibrium in some such way as the loss of water does, when the normal osmotic relations are disturbed? Or does the spermatozoon carry into the egg some specific chemical substance which produces a local differentiation, of which the centrosome and the aster are the expression? Or does it import "a highly active centrosome or centrioplasm about which the cytoplasmic energy is brought to a focus?"

Boveri (1902) holds that a general stimulation of the egg, with the sperm head as the point of predilection for the formation of the aster, as in magnesium eggs the egg nucleus is the point of predilection, is insufficient as an explanation. There is much rather something special present in the spermatozoon, which determines that the aster shall appear at that point, and that point only; and thus he thinks that still the appearances may best be described as being due to the introduction of a centrosome. Even admitting—which, as has just been indicated, he does not—that the spermatozoon acts like Loeb's agents, and in view of the demonstration by Morgan and Wilson that their effect is to cause the egg to produce centrosomes *de novo*, only a modification of secondary importance would be required in his theory of fertilisation, viz., that instead of saying that the spermatozoon brings a centrosome into the egg, it would be necessary to say that it causes the formation of a centrosome in the egg, from the division of which the rest follows.

Taking the sperm aster as the manifestation of activities produced by the spermatozoon, and looking to its sharp localisation on the site of the middle piece, it seems reasonable to suppose that the localised excitement is the effect of an agent operative in fertilisation, and that it is probably related to the middle piece; but the actual continuity between the centrosome of the spermatozoon and that in the aster has not been absolutely demonstrated, and the new facts in regard to the centrosome put the matter in another light. Thus it remains for the future to decide which of the two latter alternatives stated above shall be adopted, and perhaps after

all there is only a formal difference, for the fundamental problem is the same when the question is raised how the centrosome exercises its activities.

Loeb (1901), on the physico-chemical side, suggests that a catalytic substance is carried by the spermatozoon into the egg, that is one which accelerates physical or chemical processes which would occur without it. The K ions act as catalysers, and the loss of water acts also, though less directly, in the same way, and it may be that it gives rise to substances which act catalytically. Inasmuch as in *Chaetopterus* the normal development does not show the characteristics of a treatment of the eggs by K, it is probable that normal fertilisation is not brought about by K ions.

Delage (1901) considers that the egg is in an unstable state of equilibrium, which is readily upset by various agencies—loss of water, heat, etc., and he lays some weight on the specific action of the salts. He finds that the chloride of manganese has, for *Asterias*, a specific action superior to that of the alkaline salts. Together with his son, he showed that in the case of the sea-urchin there was less magnesium chloride in the sperm than in the eggs, by about 1 per cent., so that this salt could not have a specific action.

Among other possible factors in the action of the spermatozoon, he gives prominence to its abstraction of water from the cytoplasm. During maturation the nuclear sap is shed into the cytoplasm; until this is effected by the solution of the nuclear membrane, fertilisation is not possible; it is just at this "critical stage" in *Asterias* that he finds artificial parthenogenesis most liable to occur. In the specialisation of the sexual elements, the egg thus becomes rich, while the spermatozoon has become poor, in water. After the sperm head has entered the ovum it increases in size by abstraction of fluid from the egg protoplasm, and this abstraction of water by the sperm nucleus has to be reckoned with as a possible factor in fertilisation.

Apart from the large assumptions involved in such an hypothesis, the facts of "partial fertilisation, and the local-

isation of the aster on the middle piece, are in opposition to it."

It has been suggested that the centrosome is the seat of formation of a ferment. Mathews (1901), from the results of his experiments on the eggs of *Arbacia*, believes that "whatever the details of the process may prove to be, the essential basis of karyokinetic cell division is the production of localised areas of liquefaction in the protoplasm." "The centrosome might be a liquefying enzyme."

Experiments on this line have been tried, but without definite result. Pieri's results (1899), from which he supposed he had obtained a ferment "ovulase," have not been confirmed. Dubois (1900) showed that there was no question of a ferment being obtained by Pieri's methods. He made various experiments on sperm and eggs, from which he concluded that there was evidence of the existence of a "zymase," which he provisionally named "Spermase," in the spermatozoa, and in the egg a substance, at least modifiable by "spermase," provisionally named "Ovulase." Spermase cannot enter the egg by diffusion or osmosis, but only by a mechanical means, which is the *raison d'être* of the spermatozoon. Winkler's experiments (1900) are also inconclusive. He used sperm shaken for half an hour in distilled water and filtered five or six times through three-fold filter-paper. The filtrate was added to sea water, the precaution being taken of keeping the mixture at the same degree of concentration as the sea water. While the sperm in heated sea water produced no results, the liquid caused in the case of *Sphærechinus* and *Arbacia* eggs, though in a relatively small number, the beginnings of segmentation. These results may have been due to osmotic influences.

Loeb (1900) states that up to that date he had found no enzyme save papain which had an effect in causing the egg to segment, and it was uncertain whether this was not due to some accidental constituent of the enzyme preparation used. Gies (1901) made a complete study of the effects of extracts of sperm made by the ordinary methods for the preparation of

enzyme solutions. His results were wholly negative, and he concluded that, used in certain proportions and under certain conditions at any rate, such extracts did not possess any power of causing proliferation of the ripe ovum. No evidence could be furnished of the existence of a zymogen in spermatozoa. Extracts of fertilised eggs in the earlier stages of development seemed likewise devoid of any segmental activity. The results, Gies adds, do not, however, certainly show that enzyme action is impossible, after, or at the time of union of the spermatozoon with the ovum, within the latter.

The same negative result was got this spring by R. T. Lieper at Millport Marine Biological Station, using an extract of sperm prepared by spreading fresh sperm on sheets of glass, then drying in air and sun, and afterwards triturating the dried extract in sterilised sea water. The filtrate from this fluid produced no segmentations, though control experiments with eggs from the same ovaries normally fertilised, nearly all developed.

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The Movements and Reactions of Fresh-water Planarians: a Study in Animal Behaviour.¹

By

Raymond Pearl, Ph.D.

(Instructor in Zoology in the University of Michigan, Ann Arbor,
Michigan, U.S.A.)

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A. INTRODUCTION.

THE present study has for its purpose the analysis of the behaviour of the common fresh-water planarian into its component factors. It is well known that, aside from the researches of a few investigators on a small number of forms, we have little detailed knowledge of the behaviour of lower organisms. It is coming to be realised, too, that knowledge of what an animal does is just as important in the general study of life phenomena as a knowledge of how it is constructed, or how it develops. But it must be admitted that until quite recent times the study of the activities of living things was a much neglected field in biology. The publication of the 'Origin of Species' gave the biological pendulum a swing towards the study of phylogeny, from which it is only just beginning to return.

As a consequence of this concentration of interest on other subjects, we possess an accurate and full knowledge of the

activities of very few lower organisms. The behaviour of the Protozoa has been quite fully described and analysed by the work of Verworn ('89) and Jennings ('97, '99, '99*a*, '99*b*, '99*c*, :00, :00*a*, :00*b*, :00*c*, :01, Jennings and Moore :02). In the earlier work of Verworn the general features of most of the reactions of the Protozoa are described, special attention being paid to the rhizopods. The reactions of the Infusoria have been very thoroughly worked out by Jennings. In the case of the Infusoria we now know exactly the mechanism of the reaction to a large number of stimuli. The reactions and general behaviour in the case of two groups of echinoderms are quite thoroughly known from the early work of Preyer ('86, '87) on the starfish and the recent brilliant work of von Uexküll ('96, '96*a*, '99, :00, :00*a*) on the sea-urchin. These few instances are the only ones in the literature where the movements and reactions of an organism, or group of organisms, have been investigated in any comprehensive "monographic" way. There is a great body of literature dealing with isolated reactions in a variety of forms, but the thorough investigation of the activities of animals in a way comparable to that in which their morphology has been investigated remains in large degree yet to be done.

It appeared highly desirable that this sort of knowledge be extended, and it was with this idea in mind that this work was undertaken. The form used, *Planaria*, was chosen for several reasons. In the first place, it has come to be a sort of paradigm for work on regeneration, and its biology from that standpoint is already well known. Furthermore, in some one or more of its species it is an almost universally distributed form and can always be obtained in quantities. Finally, and particularly, it is a representative of an animal type about whose activities we know only the most general facts. It is a symmetrical aquatic organism of low organisation, and its behaviour is rather complicated. The importance of possessing a detailed knowledge of the activities of a bilaterally symmetrical, free-moving, low organism will be apparent when it is considered that such an organism has never been

made the subject of such a study. The behaviour of typically unsymmetrical organisms, the Infusoria, has been analysed, as has also that of some radially symmetrical animals, and in both cases there is found to be a very close interrelationship between the general form of the body and the reactions.

To investigate, then, in a comprehensive way the activities of a bilaterally symmetrical organism standing low in the animal series was the purpose of this work. The most general problem which presents itself is the establishment of the animal's position in the objective psychogenetic series. Are its activities relatively simple or are they complex? Do they fall under the same general type as those of the Infusoria or those of the higher organisms, or do they occupy an intermediate position? Another general problem of importance is whether there is any marked correlation between the behaviour and the form of the body, such as has been found to obtain in so marked a degree in the case of the Infusoria and the rotifers (*vide* Jennings, *loc. cit.*). We have in the flat-worm a symmetrical animal; are its reactions of a symmetrical type? Besides these broad fundamental problems there are, of course, a large number of subsidiary questions which readily suggest themselves in connection with a work of this sort. These need not be specifically mentioned here, but will be brought out in the course of the paper.

As to the scope of the work as actually done, the following may be said:—The general “natural history” of the animal was studied as completely as possible. All the normal movements were studied in detail. The reactions to mechanical stimuli; the food reactions and reactions to chemicals in general; electrotaxis; thigmotaxis; rheotaxis; the righting reaction; the reaction of cut and regenerating pieces; and hydrotaxis and the reactions during desiccation were investigated. No work was done on the phototaxis or thermotaxis. A study of the phototaxis was omitted for two reasons; first on account of the fact that during the progress of this investigation Parker and Burnett (:00) reported their work on

the same subject, and furthermore on account of lack of opportunity. As a result of some incidental observations made during the course of this work, it has appeared that it would be profitable to extend the work of Parker and Burnett, and this, together with a study of the thermotaxis, I hope to be able to do in the future. Another field for further work is afforded in the study of the reactions of regenerating individuals. As this subject did not fall immediately into the general plan of this work, but comparatively little attention has been given to it, yet the work done gives much promise of important results to be gained by more extended investigations.

So far as possible the details of the movements and reactions will be described fully. It is not easy to see why there is not as much need for a complete knowledge of details in physiological work as in morphological, yet in much of the recent work in comparative physiology only the most general results are reported. To gain a knowledge of the details one must do the work over again. While such more or less general papers are easy to read, and put the main results in such a form as to be easily accessible, yet it is believed by the writer that the solid foundations of comparative physiology and psychology must consist of detailed "fine" work, just as has been the case in morphology. It seems to the writer that the tendency to abandon the detailed descriptive method in favour of the extreme experimental method in biological work is unfortunate. Both ways of working are methods of getting at the truth, and, as proven by their results, both are good methods. The current notion of the sufficiency of the experimental method to the exclusion of others is not only an evident exaggeration of the facts in the case, but, in the opinion of the writer, the exclusive use of the "crucial-experiment" method in work upon the movements and reactions of organisms has in some cases hindered rather than helped us to gain a clear understanding of the phenomena. The importance of close observational work in the study of animal behaviour has been strongly emphasised

recently by Whitman ('99). The aim in the present work has been to get as extensive and detailed a knowledge as possible of the behaviour of the organism by direct observation before resorting to experiments.

At this point I wish to acknowledge my indebtedness to the officials of the laboratory in which this work has been done. To Professor H. S. Jennings, under whose general oversight this investigation has been prosecuted, I wish to extend my heartfelt thanks for his uniform kindness in freely giving advice, suggestion, and kindly criticism of immeasurable value. Any adequate expression of my indebtedness to him is impossible. I further wish to express my thanks to Professor Jacob Reighard for the numerous facilities which I have enjoyed during my stay in his laboratory, and for his kindly interest, which has made work there a pleasure. Finally, I desire to acknowledge my indebtedness to Professor F. C. Newcombe, of the Botanical Department of the University of Michigan, for many valuable suggestions and advice.

B. RÉSUMÉ OF LITERATURE.

But little has been done on the physiology of the movements or on the psychology of the Turbellaria, and, as in the case of most of the literature dealing with these subjects, what has been done has been in comparatively recent years. Investigators of the old "natural-history" school which flourished before the time when Darwin's work changed the course of zoology seem not to have given much attention to planarians, while the later systematists and morphologists for the most part carefully avoided any reference to the activities of the forms which they studied.

I. Morphological and Systematic.

Among the papers devoted primarily to the systematic or morphological treatment of the group, there are occasional references to points in the behaviour of the organisms which

are of importance from the present standpoint. Among such references the following may be noted :

Moseley ('74), in a paper concerned principally with the anatomy and histology of the land planarians, devotes a section to a discussion of the habits of these forms. He comments on the "avoidance of light" (negative phototaxis) of land and aquatic planarians, and discusses the habitat and food of the animals. He reaches the conclusion that all planarians are carnivorous, but gives no account of the method of feeding. He quotes Rolleston as having found that *Planaria torva* and *Dendrocœlum lacteum* in a dish in which had been placed a freshly killed earthworm "crowded on to the worm's body and soon sucked all the hæmoglobin out of it, leaving it white and pulpy." Brief mention is made of the habit of the land planarians of secreting a mucous thread and hanging from it as a mollusc does. Finally, the method of movement of *Bipalium* with the head raised and waved from side to side as the animal proceeds is described. A bibliography of previous literature is given.

In another paper Moseley ('77, pp. 273, 274) gives an account of the movements and general habits of *Geoplana flava*, a Brazilian species. This species was found to keep in shaded and moderately lighted places. The direction of the ciliary currents was tested by placing small bits of paper on the surface of the body, and it was found that when the animal was in active movement the effective beat of the cilia on the anterior part of the dorsal surface was forward and outward, while on the posterior portion of the dorsal surface the beating was backward and outward. The currents on the ventral surface were always straight backward. The author concludes that the function of the cilia on the dorsal surface is to quickly remove foreign bodies. When the organism was at rest there was no movement of the dorsal cilia; "the animal moves to a large extent by muscular action, the body alternately contracting and expanding during motion. When moving it lifted its anterior

extremity often, and moved it to and fro as if to feel or see its way." "When the anterior extremity of the body was cut off the remainder of the animal seemed still to move with definite purpose, avoiding obstacles and retreating from the light, while the cut end was raised and thrust in various directions as if to search for an object on which to climb."

In a brief note Zacharias ('88) mentions the occurrence of *Geodesmus terrestris* between the lamellæ of *Agaricus deliciosus*. Particular points mentioned are: the slow movement, characterised by the raised anterior end, and the hanging by a mucous thread after passing over the edge of a glass plate. Light stimulation of the anterior end with a needle induces a very strong contraction of the whole body.

Gamble ('93), in a systematic paper on marine Turbellaria, describes briefly the movements of a number of species of rhabdocœles and triclads.

Lang ('84), in his monograph on the polyclads, devotes a chapter to the habits, movements, and natural history of this group of planarians (loc. cit., pp. 631—641). While not done particularly from the physiological standpoint and not treating the subject experimentally, this work contains numerous valuable observations. Points especially treated are the habitat, colouration, food and method of feeding, defecation, movements, including swimming, copulation, respiration, regeneration, growth, and duration of life. The details in the behaviour of the polyclads recorded in this monograph will be discussed later in connection with the points on which they have direct bearing.

The most important paper dealing with the movements and general behaviour of planarians which I have been able to find in the literature is that of Lehnert ('91). This work is principally devoted to an account of the biology of three forms of land planarians, viz. *Bipalium kewense*, *B. kewense* var. *viridis*, and *Geodesmus bilineatus*. Besides the work on these land forms, Lehnert also made some comparative studies on several fresh-water dendrocœles and rhabdocœles. He gives an admirably full and detailed

account of the movements of land planarians; in fact, by far the best description of these phenomena in the literature. In this account the relation of the movement to the mucous secretion from the ventral surface of the body is brought out in great detail. The principal factors in producing the movement in the case of the land planarians he gives as (a) ciliary movement on the ventral surface, (b) rhythmical contraction waves passing longitudinally over the ventral surface, (c) secretion of slime, and (d) snake-like movements of the whole body. A comparison with the movements of other planarians (fresh-water) is made. In this connection it may be mentioned that Lehnert considered rhythmical contraction waves passing along the ventral surface of the animal to be a factor in the movement of fresh-water planarians (*Dendrocœlum lacteum*, *Planaria polychroa*, and *Polycelis tenuis*). This I am unable to confirm from observations on the planarians which I have studied. This point will be discussed more fully later. The food and the method of taking food in case of the land planarians, Lehnert worked out very thoroughly. They were found to be carnivorous, and in the case of *Bipalium* the pharynx was capable of being stretched over a large piece of earthworm, so that it resembled a very thin transparent skin covering it. The relations to other phases of the environment, e. g. air, water, temperature, light, solid bodies, etc., are described very briefly.

Raspail ('93), in a brief note, mentions the feeding of a planarian.

Van Duyne ('96) mentions briefly the movements of heteromorphic forms of *Planaria torva* (?). He found that the parts of two-headed individuals moved independently of each other, and that each piece would move away from the other until they had completely torn apart.

Willey ('97), in a brief note, describes the structure of a remarkable asymmetrical planarian, for which he proposes the generic name *Heteroplana*, having the left side of the body almost completely atrophied. Regarding the loco-

motion of this remarkable form he says, "In *Heteroplana* the locomotion is usually conducted in a somewhat one-sided fashion," and he furthermore figures the animal as constantly moving towards the left. It is to be regretted that no reference is made to how this form reacts to stimuli, as it would be of great interest to know whether the reactions are asymmetrical, and in general how they compare with the normal planarian type.

A series of papers by Morgan ('98, :00, :01) contains numerous references of importance on the movements of cut and regenerated specimens of various fresh-water planarians. He finds ('98), in confirmation of van Duyne, that in two-headed individuals each head tends to move in its own proper direction. In the case of a heteromorphic form with two heads pointed in opposite directions, this likewise held true; but one component being stronger this determined the movement of the whole. The lack of movement in certain forms of cut pieces is also noted. In his :00 paper Morgan notes the readiness with which "*Planaria*, sp."¹ and *Planaria maculata* take food, although no account is given of the method of the feeding reaction. An interesting observation, and one of considerable theoretical importance, is also reported in this paper. In an individual split longitudinally in the median line from the posterior end forward, in which the two parts were united only by a small connecting band of tissue at the anterior end, it often appeared "as though these pieces would pull apart, but as soon as the tension on the connecting band becomes too strong, the rest of the piece, by a sort of adaptive response, ceases pulling in its former direction." In the most recent paper cited (:01) Morgan corrects a statement of Bardeen² regarding the feeding of *Planaria*. It is maintained (and I may mention at this point that my own observations agree entirely with those of Morgan) that *Planaria* "responds freely" to food sub-

¹ Later identified by Woodworth as *Planaria lugubris*.

² To be reviewed later.

stances not actually in contact with it. This point will be discussed in detail later.

Lillie (: 01) brings out the fact that cut posterior parts of the body of *Dendrocœlum lacteum* show very little movement, and in general fail to give the typical reaction to light after removal of the brain.

Finally, there are accounts of the natural history and habits of various planarians in numerous "natural histories" and text-books. As such accounts are for the most part brief and of no great significance from our standpoint, they will not be referred to in detail.

II. Physiological.

The literature dealing with the planarians from a purely physiological standpoint is very meagre. Furthermore, for the most part it deals only with special phases of the physiology of these organisms, there being very little work attempting to bring the behaviour of planarians into relation with that of other forms.

The most important work dealing experimentally with the physiology of the movements of flatworms which I have found is that of Loeb ('94). The purpose of his work was to determine in how far the reactions of such low organisms as worms were dependent upon the brain. The planarians used were *Thysanozoon brocchii*, and *Planaria torva*. In *Thysanozoon* he found that if the animal were quickly cut into two pieces transversely with a sharp scalpel or scissors the anterior piece crawled on undisturbed, while the posterior piece showed no further movement. The conclusion is then drawn that "Die Spontaneität der Progressivbewegungen ist also bei *Thysanozoon* eine Funktion des Gehirns." This form shows no definite "geotropic" reaction, but crawls about with the axes of the body forming any angles with the direction of gravitation. The very strong "stereotropism" (thigmotaxis) of the ventral side of *Thysanozoon*, which always tends to keep in contact with a solid body, is noted. This reaction is found to be inde-

pendent of the brain. There was found to be co-ordination between the anterior and posterior pieces of a worm in which the lateral longitudinal nerves had been cut, but in which a narrow connecting strip of tissue had been left between the pieces. In *P. torva* Loeb states that posterior parts of the body which have been separated by a transverse cut from all connection with the brain crawl "ebenso munter weiter, wie die orale Hälfte." The reaction of this form to changes of light intensity is discussed in considerable detail, it being shown that in strong light the organism is stimulated to active movement, while in the shade it remains quiet or moves very slowly. This was found to occur as well in decapitated as in normal worms. The "stereotropic" reaction in this form is also mentioned. In concluding, the author holds that in worms there is no "associative Gedächtniss," and hence no consciousness. These results have been recently incorporated without essential change into a larger work (Loeb : 00).

In an earlier paper Loeb ('93) first described the reactions to light of *Planaria torva*. These results were incorporated without essential change into the '94 paper mentioned above.

Hesse ('97), in his morphological studies on the eyes of flat-worms, devotes a section to the subject of the reactions to light of *Euplanaria gonocephala* and *Dendrocoelum lacteum*. His results are confirmatory of Loeb's, nothing of particular significance being added.

Steiner ('98) found that posterior pieces of *Planaria Neapolitana* (= *Stylochus pilidium*, Lang) separated from the brain by a transverse cut would move about freely after recovery from the operation. He believes this ability to move is conditioned by the presence of ganglion-cells in other parts of the body than the brain (along the lateral nerve-cords).

Parker and Burnett (: 00) have recently made a thorough study, using very careful experimental methods, and treating the results statistically, of the reactions of *Planaria*

gonocephala to light. This form moves away from the source of the light. The amount of directive influence was measured. It was found that specimens without eyes, i. e. in which the anterior end had been cut off, react in much the same way to light as do normal individuals, "in that they have a tendency to turn away from the course when directed towards the source of light, and to keep in it when directed away from the source, though with less precision, and often to less extent, than planarians with eyes." Furthermore, figures are given showing that planarians from which the anterior end has been cut off move more slowly than normal animals. This is thought to be due to the absence of the eyes.

The most extensive paper dealing with the physiology of planarians is that of Bardeen (: 01). This paper is mainly devoted to a study of regeneration in *Planaria maculata*, but before entering upon the discussion of this subject the author devotes considerable space to an account of the anatomy and physiology of the organism. In the section devoted to physiology, the author discusses, under the caption "Environmental Activities," sensation, movement, and the central nervous system. The author makes the remarkable, and obviously incorrect, statement that the planarian is sensitive only to light and contact. A very few inconclusive experiments having reference to thigmotaxis, geotaxis (?) and hydrotaxis, are reported. The statement is made that specimens "would remain unmoved by the presence close by their side of a piece of fresh snail, a food much prized by them." Two forms of movement are described—"swimming" and crawling. The author's description of what he calls the "swimming" movement will be discussed later in this paper. Brief and very general statements regarding the reactions to mechanical stimuli are presented. Under the heading "Internal activities" are discussed deglutition, food dispersion, defecation, and respiration, in a rather loose and hypothetical way. The author makes the following contribution regarding excretion in *Planaria*:—

"Excretion is carried in part through the intestines by the act of defecation; in part it is doubtless carried on by an excretory system opening on the surface." A more detailed discussion of various points raised by Bardeen will be entered into in connection with the parts of this work on which they have direct bearing.

A second paper by the same author (Bardeen, :01a) describes briefly the normal food reactions of *Planaria*, and shows that a decapitated specimen will not find food material in a dish, although one such a specimen could "be made to eat if it were placed on its back on a slide in a small drop of water. Under the conditions mentioned the pharynx is usually protruded, and will engulf bits of food placed in the mouth." An experiment was performed in which the part of the head in front of the eyes was cut off. Such specimens, from which merely the tip of the head had been removed, reacted normally to food. It is also shown that specimens from which the part of the body posterior to the pharynx has been removed feed like normal worms. Regarding the method by which planarians find food in their immediate vicinity, Bardeen says (p. 176), "It is difficult to determine the source of the impulse which gives rise to this purposeful activity. It is possible that the auricular appendages here act as delicate organs capable of stimulation by slight currents in the water set up by the minute organisms that prey at once upon the flesh of the dead snail." Experiments to be reported in the course of the present paper show, I think, that the mechanical and chemical stimuli given by food are the ones which affect planarians.

C. MATERIAL.

The following species have been principally used in this study:—*Planaria maculata*, Leidy; *Planaria gonocéphala*, Dugès; *Planaria dorotocephala*, Woodworth.¹ Of these *P. dorotocephala* and *P. maculata* have been

¹ Excellent figures and descriptions of these three species have been published by Woodworth, '97.

most used, both on account of their abundance and, furthermore, because *P. dorotocephala* is a form particularly favourable for the study of reactions. It is very active, and after being disturbed continues in movement longer than either *P. maculata* or *P. gonocephala*, as has already been noted by Woodworth (loc. cit., p. 7). I have found also that it moves faster than either of the other two species. There is a general precision and positiveness of response in its behaviour which make it especially favourable for experimental work. A large number of experiments have been made with a view to determining whether there was any difference in the reactions of these three species, but no essential difference has been found. The form of the reactions is the same in all cases. Whatever differences there are are differences of degree, such as would be conditioned by the relative sluggishness and activity.

Certain forms of reaction to mechanical stimuli, and to chemical stimuli, are rather more easily induced in *P. dorotocephala* than in either of the others, yet, as will be shown later, these reactions will be given, under the proper conditions, by the other species. This being the case, and since *P. dorotocephala* was, for reasons noted above, most used in this work, it will be employed throughout the paper as the type form, and it will be understood, when there is no statement of the species, that *P. dorotocephala* is the form meant.

No account of the anatomy of these forms will be given here, because it has been very fully treated in other readily accessible papers. The most important papers dealing with the morphology of the fresh-water triclads are those of Jijima ('84), Lang ('81, '81a), Kennel ('88), Chichkoff ('92), and Woodworth ('91 and '97).

Besides the species mentioned above, on which the most of the work was done, a number of observations and experiments have been made on several other species of triclads and rhabdocæles. The other triclad most frequently met, and whose reactions have been found to agree closely with

those of the species of *Planaria*, is a form which agrees with the description of *Dendrocœlum lacteum*, except in respect to the colour. This form is usually coloured from a light grey to nearly black. The colouring is uniform. In only one specimen have I found any deviation from this typical coloration, and in that case there was a band of black pigment extending the whole length of the body along the mid-dorsal line. In width this band occupied about one third the whole width of the body. The margins were a pure white, without the faintest trace of pigmentation. This specimen was kept under observation for some time, and there was no doubt that it belonged to the same species as the grey form. The specimen struck one at once as being transitional between the ordinary white to cream-coloured *Dendrocœlum lacteum*, and the grey form found about Ann Arbor. Being in some doubt as to the true taxonomic position of this grey species, I shall refer to it throughout this paper as *Dendrocœlum*, sp.

Besides the forms mentioned, several undetermined triclads have been collected and worked with to some extent, but as no new factors presented themselves in their reactions they will not be considered in this paper.

A large rhabdocœle, which I have identified as *Mesostoma personatum*, O. Schm., is found rather commonly in certain localities about Ann Arbor in the spring. I have done some work on this form. Another rhabdocœle whose actions I have studied to some extent is *Stenostoma leucops*, O. Schm. No detailed investigation of the behaviour of the rhabdocœles was made, but as opportunity offered they were used for comparison.

The methods used in experimentation will be given under the separate headings dealing with the reactions.

D. HABITS¹ AND NATURAL HISTORY.

In the course of more than two years' study of planarians

¹ In this section the word "habit" will be used to signify merely those activities of the organisms which are frequently observed to occur under

numerous observations have been made on their general natural history. It is thought desirable to present a general account of this here for two reasons: first, because there is no adequate discussion of the natural history of the fresh-water trielads in the literature; and furthermore, because it will bring out prominently the phenomena for which we are seeking an explanation. In other words, it will present the problems with which this study has had to do.

I. Occurrence and Distribution.

The species of *Planaria* (*maculata*, *dorotocephala*, and *gonocephala*) used in this study have been collected mainly from the Huron River near Ann Arbor. They are found, for the most part, on the under surfaces of stones in places where the current is of moderate swiftness, and the substrate on which the stones rest is rather soft. They are also found among the fronds of such water plants as *Ceratophyllum* and *Elodea*, although less abundantly than on stones. I have obtained these species only very rarely in collections from ponds and small pools of stagnant water. They appear to be, in general, much more abundant in shallow water than in deep.

Rhabdocœles I have found in great abundance in small ponds and pools of stagnant water, and, with the exception of *Stenostoma leucops*, almost never in running water. *Dendrocœlum*, sp., is also much more abundant in stagnant water than in streams.

There is no marked seasonal distribution of the species of *Planaria* studied. They appear to be slightly more abundant in the fall than in the spring. I have found no evidence of any migration into deep water during the winter in the case of these forms, as has been described by Child (:01, pp. 978—981) as occurring in *Stichostemma*. The seasonal distribution of *Dendrocœlum*, sp., appears to be well marked,

natural conditions, without necessarily implying the same idea as that embraced in the term "habit" as used by the psychologists.

individuals being found in considerably greater numbers in the spring than at any other time in the year so far as my observations go. This seems to be true also of the rhabdocœles.

Relatively the most abundant species of planarians in this region are *Planaria dorotocephala* and *maculata*. The numbers of these two are about equal, with a slight advantage in favour of *P. dorotocephala*. Next in abundance I have found to be *P. gonocephala*, but this is considerably below the other two. Finally comes *Dendrocœlum*, sp., which I have never found in an abundance to be compared with the species of *Planaria*.

II. Activities.

The movements of planarians will be discussed in detail in a later section of the paper,¹ but it is desired to take up here certain general activities and relations to the environment which properly fall under the general subject of natural history.

The first of these subjects is—

a. Sensitivity.—The flat-worm is extremely sensitive to a variety of stimuli. Among the different stimuli which produce specific reactions, and to which we must therefore conclude it is sensitive, are the following:—Mechanical disturbances of the general environment (shaking, jarring, movement of water, etc.), contact (localised mechanical stimulation), chemical changes in the environment (in the widest sense, including food substances), light, the electric current, desiccation, a current of water, and heat.

Its extreme sensitivity, which makes it responsive to very slight changes in the environment, may be shown by a very

¹ It may be stated here, for the convenience of the reader before reaching the full discussion of the movements, that the progressive movements of triclads are of two sorts. These are (*a*) gliding movements, in which there is little or no muscular action; and (*b*) crawling movements, in which the motion is effected by muscular contractions involving the whole body. The crawling has some general resemblances to the method of progression observed in a leech of the genus *Clepsine*.

simple experiment. If a dish containing specimens not in any way stirred up by rough handling, but gliding along the bottom, be jarred ever so slightly, every individual will instantly stop, contract, and remain immovable. If only a single jar is given, the worms will start after only a momentary pause. A further experiment shows more strikingly the same thing. If in a dish containing water to a depth of not more than 1 to 1.5 cm. a single specimen gliding quietly is selected, and a needle is touched to the surface of the water above or to one side of it, it will be seen, if closely watched, to give the same momentary pause and partial contraction. If the needle is pushed down through the water towards the worm in any but the quietest and gentlest way the contracted state will continue. Only at the moment when quietness in the surroundings intervenes again will movement be resumed. I have frequently tried to introduce a needle close beside the animal without causing this momentary pause. With a layer of water not over a centimetre in depth covering the worm I have not been able to do this, except in rare instances. After the point is once through the surface film it may be brought nearer the worm without causing a persistence of the contraction, provided it is advanced in line with itself, i. e. not slid up laterally. In order to observe this extreme sensitiveness to disturbance of the water one must take care that the animals have not been violently disturbed just previously. Any marked disturbance or persistent, more or less violent stimulation puts the animals in a condition which may be called, for lack of a better term, "excited." Such a condition is characterised by increased rapidity of movement and increased general activity, and in this condition the animals do not give the "finer" responses,—that is, responses to weak stimuli. I shall have occasion to discuss this matter in more detail later.

This marked sensitivity and its associated behaviour are remarkably similar to what has been found by Whitman ('99) to obtain in the case of the leech *Clepsine*. He has further pointed out that lack of attention to this extreme sensitivity,

which is apparently quite generally distributed among lower organisms, may be an important source of error in work on the behaviour.

Regarding the statement of Bardeen (: 01, p. 14) that he does not find that *Planaria* "is sensitive to anything but light and contact," nothing need be said here. The detailed accounts of the reactions of the organism to a variety of stimuli which follow in this paper are in themselves a sufficient criticism.

b. Secretion of Mucus.—There is secreted at all times over the surface of planarians a sticky slime, apparently of the nature of mucus. This secretion is increased when the animal is irritated, and is under normal conditions more abundant on the ventral than on the dorsal side. If a needle or fine glass rod is touched several times on the surface of the body its end becomes covered with this secretion. For this reason it is necessary in applying localised mechanical stimuli to wipe the mucus off the end of the needle frequently, in order to obtain good results. Similarly, if one is using a sharp scalpel to cut the animals, if the edge is left in contact with the surface of the body any length of time before the decisive cut is made, the edge will become so coated with mucus that a clean cut is impossible; instead, the animal will slip from under the knife.

When the animal moves about it leaves behind a more or less heavy string of this mucus, so that if several specimens are placed in a clean glass dish the bottom will, in a short time, become covered with a network of interlacing mucus threads. The same phenomenon occurs in other *Turbellaria* and among the *Nemerteans* (cf. Child, : 01, and Wilson, : 00). The threads when first secreted are so transparent as to be invisible, but in larger quantities they appear opalescent, and may be picked out of the dish with forceps.

The function of this secretion in locomotion is evidently to attach the body to the substrate. Secretions for such a purpose occur widely among aquatic organisms.

The mucus also undoubtedly plays an important part in the attachment of the animal to the under side of the surface film. When the worm leaves the surface film in open water, i. e. when it cannot reach any solid body, it hangs by the mucus thread in much the same way that a terrestrial mollusc, like the common slug, does when it passes through the air from a higher to a lower point. This observation I have made many times, though generally in an indirect way. As has been said before, the mucus thread is invisible when first secreted, so that when a worm leaves the surface film it seems to glide freely through the water. If, however, one passes a needle horizontally through the water immediately above the posterior end of a worm which has just left the surface film, it will be seen that at a certain point (where the needle strikes the thread) the end of the worm will be jerked to one side. Furthermore, one may with care pick up the invisible mucus thread with forceps and raise the whole worm, provided the attempt is made before the anterior end reaches the bottom. I have seen specimens of *P. maculata* crawl back upon the thread after going a part of the way down to the bottom, and again regain a position on the surface film. The same thing is frequently done by slugs. When the animal has fully reached the bottom, connection with the thread which has served to suspend it in the water is usually broken by several sharp jerks of the posterior end of the body from side to side.

The relation of the organism to this slimy secretion is much the same in the land planarians, according to the observations of Lehnert ('91). He distinguishes in case of these forms "Kriechfaden," "Brückenfaden," and "Gleitenfaden" formed from the slime, the distinction being based on the relation of the thread to the surroundings. The "Kriechfaden" are the threads left behind as the organism moves over a continuous solid body, and the "Gleitenfaden" are the threads on which the animal hangs in passing through the air from a higher to a lower level. Both these forms of threads I have found in case of the fresh-water

planarians. The "Brückenfaden," which are formed by the land planarians when they pass from one solid body to another at about the same level, I have never observed in case of fresh-water planarians, though I see no reason why under proper conditions they would not be formed. Lehnert (loc. cit., p. 17) says, "Die Wasserplanarien bilden wie die Landformen ihren Kriech-, Brücken-, und Gleitfaden." He also noted that *Polycelis tenuis* was able to crawl back upon a mucus thread after passing for some distance down over it.

Nothing like the formation of "cysts" from this mucus, such as Child (:01, pp. 989 to 993), found in the case of *Stichostemma*, has been observed in the case of planarians. Its only biological significance in these forms is in relation to movement, as pointed out above.

In connection with the subject of mucus secretion it may be well to point out the tenacity of the attachment of the flat-worm to the bottom. It will be found in attempting to dislodge the animal that the extreme anterior end and the extreme posterior end stick very firmly to the substrate. Whether this holding is the result of a sucker-like action of the ends of the body, or is due merely to the stickiness of the mucus, I have been unable to decide. It is easily possible that the muscles could be so contracted as to form out of either end of the body a practical sucker, but whether this is done or not it is impossible to say. Woodworth ('97) has described a permanent anterior adhesive disc in *Dendrocoelum lacteum*, but considers that this "is not a true sucker, nor does the animal employ its anterior end for the purpose of attachment to any greater degree than the posterior or lateral margins of its body, along the ventral surface of which numerous mucus glands have their openings. In truth, it is the margins and posterior end that adhere more firmly to a support; often when the animal is forcibly removed from the sides of the aquarium the parts of the margin or the posterior end will adhere so firmly to the glass that the points of attachment are drawn out into

digitate processes." I incline to the view that in *Planaria* it is the mucus which attaches the organism to the support, although it must be said that the appearance is at times strikingly as if the anterior and posterior ends acted as suckers.

c. Periods of Activity and Rest.—There is in the case of freshly collected planarians a certain periodicity in the activities. First, there is the rather marked difference in the amount of activity in the night and day. It has been stated by a number of investigators that planarians were probably nocturnal in their habits, i. e. more active at night than during the day. This can easily be seen to be the case in the following way:—In a dish containing a large number of planarians, together with some plant material like *Ceratophyllum*, usually comparatively few specimens will be seen during the day. Nearly all will be in among the fronds of the plant material in a quiet condition. If, however, one comes into the laboratory at 8 p.m. or later at night, so that (in case of winter days) there has been two and a half or more hours of darkness, a large number of the specimens will be found on the sides and bottom of the dish in active movement. Again, one will frequently find in the morning that the specimens are scattered about all over the sides and bottom of the aquarium dish at rest. By noon many of these will have disappeared, or, in other words, gone in among the plants, where they are protected from the light.

Besides this day and night periodicity there is another fact that may be mentioned; this is that during the day, at any rate, they seem to be incapable of continuing movement more than a certain, not very great, length of time. Then a period of rest must intervene. Thus one may see a specimen which has been moving about come to rest, and after a length of time, varying from a comparatively few minutes to several hours, it will start into spontaneous movement again, and repeat the whole cycle over and over. It seems that the periods of quiet are really for the purpose of

resting, i. e. the animal seems to be quickly fatigued by its own movements. This is indicated by the fact that if one stirs up a specimen, and sets it into activity again just as soon as it comes to rest, the periods of spontaneous activity will become progressively shorter, until finally the worm will only move a very short distance before coming to rest again. The periods of activity are longer and more frequent in *P. dorotocephala* than in any of the other species I have studied.

d. Formation of Collections.—There is a well-marked tendency for specimens of planarians to form well-defined groups or collections when they come to rest on an open surface like the bottom or sides of a glass dish, or on the under side of rocks, under natural conditions. Of course,

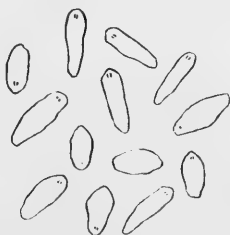


FIG. 1.—Diagram showing the appearance of a collection of resting planarians.

this is in part a result of their reaction to light, as has been noted by Loeb ('94). Besides this there seems to be some other factor at work, for in the same dish one frequently finds several localised collections from one to two inches in diameter in different parts of the dish. In these collections the specimens may be closely packed together, and with some specimens overlapping and lying partly over others, yet in the species I have studied a looser arrangement of the character shown in fig. 1 is the more usual one. On the under surface of stones such groups are frequently seen; two or three may be found on the same medium-sized stone. In this case light as a factor cannot be present, since the conditions of, all with reference to this stimulus are equal.

We have, then, here a case of what appears superficially to be "social instinct."

c. Movement on Surface Film.—As is well known, flat-worms and a number of other animals frequently move about on the under side of the surface film at the top of the water. On account of the flexibility of the support, motion under these conditions is very slow, and usually, after having been on the surface film for a short time, the worm will loosen its hold and pass down to the bottom in the way which has been described above. The worms do not remain customarily in the angles formed by the surface film with the side of the dish, as does *Stichostemma* (Child, :01), but instead pass out at once on to the free surface. Further, the flat-worms never push through the film at the side of the dish and pass up out of the water as the nemertean does. The occurrence of planarians on the surface film is not the result of any thigmotactic reaction (using thigmotactic in the sense ordinarily understood), but is brought about by a simple reflex act, and is the result of the configuration of the surface of the water and the side of the dish. This will be brought out in more detail later. It is probable that fresh-water planarians, in their normal habitat, very rarely take up a position on the surface film. Among other organisms (*Eutomostraca*, *Hydra*, etc.) this habit probably has a much greater biological significance than in planarians (cf. Scourfield, '94, :00, :01). When on the surface film the worm behaves in nearly every respect as it does when on the bottom. The head is frequently raised (with reference to the worm) and waved about in the water just as occurs in the normal movement. That the situation is a more or less abnormal one, however, is shown by the fact that, so far as I have observed, the worm never comes to rest on the surface film, but instead, always keeps in active movement till it leaves it.

The means by which the animals maintain their position on the under side of the film is undoubtedly the mucous secretion from the ventral surface. This is very sticky, and holds

the animal to the film, the surface tension being sufficiently great to support a considerably greater weight than that of a flat-worm.

It is interesting to note in this connection that the land planarians are able to move about on the top of the surface film of water to a limited extent (cf. Lehnert, loc. cit., p. 16). The immediate means of support here, as in the case of the fresh-water planarians, is the mucous secretion.

The leaving of the surface film by means of the mucus thread described above apparently does not take place if it is possible for the same result to be accomplished in any other way. Before it occurs the worm usually stretches the anterior end down into the water, and turns it in all directions. If it comes in contact with something solid the anterior end becomes attached to this, and pulls the posterior end of the body away from the film. If nothing solid is within reach the worm will usually, after a time, drop down on a mucus thread as described.

III. Food.

Planarians will take almost any sort of animal food very readily. I have used mainly, in the feeding experiments, crushed pieces of fresh-water molluscs, such as *Physa*, *Planorbis*, etc. One of these molluscs, removed from the shell and placed in a dish containing a large number of planarians, will, in a short time, be literally covered with the worms feeding. If a worm is gently lifted off the pile the greatly stretched pharynx will be brought into view. The worms will eat any other kind of animal tissue (fresh meat, parts of insects, pieces of fresh-water worms, etc.), so far as I have observed, the only condition being that the meat must be fresh. As will be shown later, the juices from the food act as chemical stimuli, so that it is necessary that the tissue be crushed or bruised so that its juices can escape into the water. A partially crushed specimen of *Planaria*, even though still able to move about, will be seized upon and eaten

as quickly as any other food. I have several times seen specimens thus eaten. It is, in fact, possible, with a little patience, to make a specimen eat a small piece cut off the posterior end of its own body! This eating of each other does not occur, so far as I have observed, unless an individual is bruised so that some of the tissue underlying the epidermis is exposed. Under these conditions juices escape from the body and act as stimuli on the other worms. Under normal conditions contact of one individual with another does not start the feeding reaction, which is a purely reflex phenomenon, capable of being started only by a certain set of stimuli. Promiscuous cannibalism, such as Child (:01) suspects to occur among individuals of *Stichostemma*, I have seen no evidence for among the Turbellaria.¹

In the feeding the worm lies fully distended, with the posterior two thirds of the body on the meat, or whatever else is being used for food; the pharynx is extruded, frequently to nearly half the length of the body, and its end is attached to the meat. During the feeding the very anterior end of the worm is attached to the bottom of the dish, provided the piece of food is not so large as to make this impossible.

Besides the animal food which the worms will take so readily, they also normally, probably to some extent, feed on vegetable matter, although I have not been able to induce the typical food reactions (to be discussed later) with vegetable material. The evidence for the statement that vegetable food is used by planarians is of two sorts: (a) specimens are frequently found extended on the stalks of water plants, with the pharynx extruded and attached to the stalks; and (b) the faeces which have been observed immediately after defecation have been found to consist largely of finely divided plant tissue. It would appear, however, that

¹ Bardeen (:01, A, p. 176) says, "Strong planarians often prey upon weak ones. In such instances the strong individual attaches its pharynx somewhere upon the body of the weak one, usually near the head." I have never seen even the largest specimens eat smaller ones unless these latter were bruised in some way.

vegetable food is not alone sufficient to keep the animals in good condition, for specimens kept in an aquarium dish with plenty of living plant material, on which they stay the greater part of the time during the day, will steadily grow smaller unless animal food is given them.

The food is in part digested, or at any rate softened, and physically changed outside the body. A piece of mollusc, on which a number of worms have been feeding for some time, has a white, fluffy appearance, similar to that of meat after partial gastric digestion. This is apparently brought about by a secretion poured out of the end of the pharynx. The necessity for some such action is apparent, because the flat-worm has no teeth or other means of separating a portion of ordinary tough fibrous tissue off from a mass so that it can be swallowed. This can be done, however, if the mass is first softened and partially dissolved. There are certain other evidences that a secretion is poured out from the pharynx during the feeding process. These will be taken up in another connection.

After the worms have fed undisturbed for a certain length of time they will leave the meat, and, after a short period of activity, come to rest.

The worms are able to live for a considerable length of time (at least two months) without food, although they continually grow smaller during this time. This marked decrease in size while starving has been noted by several observers, and especially studied by Lillie (:00). This author finds that the decrease in size is accompanied by a simplification of structure—a sort of “development backward,” such as has been described by Patten ('96) for abnormal embryos of *Limulus*.

IV. Defecation.

The process of defecation has been observed by Bardeen (:01). The process consists of three or four general contractions involving the whole body, during which the

contents of the intestine can be seen to be in rapid motion. Soon after the beginning of the contractions, which are in character different from any other of the movements of the body which I have observed, and which cannot be adequately described, the intestinal débris is shot out of the pharynx. The force of the expulsion is so great that the faeces spread out in the water some considerable distance from the opening of the pharynx. I have observed the process only a few times ; apparently it occurs only at infrequent intervals.

V. Summary of Factors in Behaviour.

From the above sketch the behaviour of the flat-worm can be seen to be of considerable complexity. The movements show many variations in character, rate, and direction. The animal shows apparent preferences for certain situations while avoiding others. It reacts to a variety of stimuli in ways which, on the whole, further its preservation and well-being perhaps as well as if guided by careful thought. It chooses its food, taking certain things and passing by others. It forms gatherings of a sort which apparently indicate that the flat-worm prefers to be in the company of his fellows ; in other words, it seems to have something of "social instinct." On the whole, as the further analysis will show, it fits itself to its environment by its activities in a way which would not be discreditable to a being possessed of considerable powers of reasoning.

Our problem now is to analyse, as far as possible, this complex behaviour into its component factors. Each activity will be taken up in detail and subjected to thorough scrutiny, to determine its essential nature, and whether it may not be resolved into simple components. With this analysis completed, it will be possible to assign the organism a definite position in the objective psychological scale. With the internal psychological factors—those of which there is no objective criterion—we shall not attempt to deal. The purpose of the paper is to furnish the data which may be

obtained by an objective study of the phenomena: precisely what these imply as to internal factors would doubtless be a subject of dispute among psychologists of different schools.

E. NORMAL MOTOR ACTIVITIES.

Under this heading will be included all the purely motor phenomena of the organism. This will include the movements (without reference to special reaction to stimuli), the coming to rest, and the general resting condition of the organism.

The movements naturally fall into two categories; (*a*) locomotor movements, and (*b*) non-locomotor, including such movements as contractions and expansions and the like.

I. Locomotor Movements.

As has been mentioned above (p. 19), there are two sorts of locomotor movements, the gliding and the crawling. The gliding is the smooth, even motion by which the flat-worm slips about over surfaces without showing a perceptible ripple of muscular movement. This is the characteristic movement when the organism is not particularly stirred up. The crawling is the characteristic movement when the animal is, or has been recently, strongly stimulated. It is a purely muscular movement.

a. Gliding.—The movement which I have called gliding is apparently the same as that which has been called "swimming" by Bardeen (*loc. cit.*, p. 15), yet it must be stated that in all of my observations on a very large number of planarians I have never seen anything corresponding to some of the details which this author mentions in this movement. In the first place, he speaks of the worms moving progressively when not in contact with a solid body, i.e. of a movement freely through the water. This I am unable to understand, as I have never seen the slightest indication of the organism moving freely in the water without contact with a solid body or something which served the purpose of

a solid (viz. the surface film). The only possible exception to this is the passage of the animals from the surface film to the bottom on a string of mucus, as described above. Furthermore, so far as I can find in the literature, no one else has ever seen a fresh-water triclad swim freely through the water.

The movement takes place with the body in contact with a surface either of a solid or of the surface film. There is, of course, between the ventral surface of the body and the surface on which it is moving, the thin layer of mucus which is constantly being secreted. It is in this mucus layer rather than the free water that the cilia beat.

This gliding movement is, so far as I have been able to ascertain, brought about by the action of the cilia on the ventral surface. There may also be some very slight muscular movement of the ventral body-wall comparable to that in the foot of a mollusc like *Physa*, which assists in the locomotion; but in the case of the flat-worm this factor, if it exists at all, is very insignificant. Only in a few instances have I been able to satisfy myself that any such movement was taking place, and then it did not have the characteristic rhythm seen in a mollusc. If this factor has any effect at all on the gliding movement it must be an extremely slight one.

The cilia beat strongly backward, i. e. towards the posterior end of the body. I have not been able to induce any reversal of the ciliary beats in these ventral cilia. Bardeen (loc. cit., p. 15) states that when the head is suddenly drawn back from some object the movement of the cilia on the antero-lateral margin of the head is reversed, and further suggests that "this reversed action may possibly be set up by the mechanical friction of the water." It would appear that the suggestion is the correct explanation, and that this is not a true reversal of effective beat.

The cilia which are mainly effective in producing the gliding movement are distributed on the ventral surface of the body, as shown in Fig. 2. There is a band down the centre of the body, which widens out at the anterior end so

as to cover nearly the whole of the ventral surface of the head. The beat is the strongest down the median line of this band, and diminishes in intensity towards either edge until at the margins there is no ciliary movement at all. At the anterior end the cilia near the side of the head beat backwards and at the same time inward towards the median line, so that the currents take the course indicated by the arrows in that region in Fig. 2. The distribution and action of these cilia were made out by stirring finely powdered indigo in the water, and then either directly observing the ciliary action on these suspended particles as the animal glided on the surface film, or by indirectly observing it in a mirror placed below the bottom of the glass dish in which the worms were. Both of these methods gave the same results,

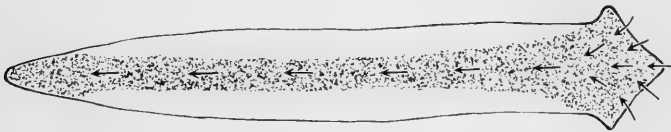


FIG. 2.—Diagram of the ventral surface of *Planaria*, to show the distribution of cilia. The stippled area is that which bears cilia. The arrows indicate the direction of the ciliary currents. (The pharynx is omitted for the sake of clearness.)

and showed very clearly the distribution of the effective cilia.

I have found no evidence of ciliary action on the dorsal surface of the body. Around the margins of the head there are cilia, but in other parts of the body, either on the dorsal surface or the edges, I have found no evidence of their presence. Particles of indigo dropped on the dorsal surface of a worm will remain in the same place for hours at a time. This is in striking contrast to the conditions in the land planarians as described by Moseley ('77), where the dorsal surface is thickly covered with cilia, which serve the purpose of keeping the body freed of foreign matter.

In the gliding movement the head is raised slightly from the bottom so as to form an angle with the rest of the body.

This position is shown in Fig. 3. As will be brought out later, the head is a particularly sensitive portion of the body, and apparently its elevation is related to its sensory function, in that it practically brings the head into close relation with a large environmental field. The head is not held in a fixed raised position, but is in constant though slight movement whenever the animal as a whole is moving. These "feeling" movements ("tastende Bewegungen") of the head are very characteristic. The head as a whole is raised and lowered, and turned from side to side, while at the same time the antero-lateral margins are moved up and down and extended and retracted. These "feeling" movements of the head region are usually very slight, and escape notice except under close observation. When the organism is much stirred up, however, they may become quite apparent. Their purpose is evidently to increase the chances of receiving stimuli, so that any stimulus in the neighbourhood



FIG. 3.—Diagrammatic side view of a gliding planarian.

may be quickly received. Constantly different sensory surfaces are presented to the environment. The head region acts in movement as a single great tentacle-like organ which is constantly testing the environment as the animal proceeds. At the same time the auricles are fully extended and raised. I do not think that this marked sensory activity functions so much for the protection of the organism against harmful environmental influences as it does to give prompt notice of useful stimuli,—for example, stimuli due to the presence of food material. In general it would not appear that such an organism as the flat-worm runs as great risk of elimination from enemies as it does from not finding food material for its own support. In the ctenophore *Mnemiopsis leidyi*, whose reactions I have studied, no trace was observed of a reaction adapted to the purpose of getting the organism out of dangerous surroundings, but its only specific

reaction is one which would bring it towards any food material which might be encountered.¹ While, as will be shown later, there is in the case of *Planaria* a reaction which is adapted to getting the organism out of danger, yet it is not called forth by so weak a stimulus as is the food reaction, and it is evidently for the purpose of receiving stimuli of the lowest intensity that the "feeling" movements are adapted.

In addition to the slight "feeling movements" of the head, described in the preceding paragraph, the organism frequently in the course of its gliding raises the whole anterior part of the body off the bottom and waves it about in the water. The portion of the body so raised may include the whole anterior half. The gliding is usually entirely stopped or very much decreased in rate while these waving movements are taking place. The head is swept from one side to the other and raised high in the water, covering a considerable area. This movement is also undoubtedly for sensory purposes.

In the gliding movement the body back of the head is kept in an approximately straight line; that is, there is no sinuous bending of the body such as is observed, for example, in *Stichostemma* (Child, loc. cit., p. 981), or at times in the movement of the earthworm. Furthermore, I have never observed any regular undulation of the margins of the body during movements such as take place in case of many polyclads, e. g., *Leptoplana tremellaris* (cf. Lang, '84). Bardeen (loc. cit., p. 15) seems to imply that such motions occur, and are an aid in the locomotion, but I am unable to confirm this statement. There are, of course, slight movements and changes of contour of the margins of the body, but they are not of a prominence or character to warrant thinking that they in any way contribute to the propulsion of the animal. In fact, it seems more probable that they are in part passive results of the motion of the whole body, and

¹ A brief preliminary account of the reactions of *Mnemiopsis* has been published in 'Science,' N. S., vol. xii, No. 311, pp. 927, 928, : 00.

in part the expression of local changes in the tonic contraction of the muscles.

In the gliding movement the body is in close contact with the surface along which the animal is moving. When an animal passes from the resting condition into movement one can see the body lengthen and flatten so as to hug the surface. By observing with a compound microscope an animal gliding along the vertical side of a dish so that the edge is brought sharply into view, the closeness of the contact of the margin of the body with the surface can be well seen. Furthermore, in specimens in which the posterior part of the body has been split longitudinally in the middle line to a point just behind the head, it is found that the half of the body which is determining the direction of the movement is always in close contact with the surface, while the other half only lightly touches it.

It would appear from all the observations which have been given that the gliding movement is brought about in the following way:—The ventral surface of the body constantly secretes mucus in greater or less quantity. This mucus can be shown experimentally to be very sticky immediately after it is secreted into the water. As it is secreted under normal conditions it immediately sticks to the surface on which the animal is reposing. Thus there will be constantly between the animal and the surface on which it is moving a layer of mucus which is adherent to the substrate. We can think of the lowest part of this mucus layer where it is stuck to the surface as of denser consistency than its upper layers which are in contact with the animal. In this upper layer of the mucus the cilia are beating and constantly pushing the animal forward. Of course, what really takes place is that the cilia are pushing the secreted mucus backward, but as this layer of mucus becomes fixed to the substrate as soon as it is secreted, the practical result is that the animal is pushed ahead. This relation is shown in Fig. 4. A represents a side view of a gliding worm; D is the substrate; C the cilia on the ventral side of the organism; and B the mucus secre-

tion, represented disproportionately exaggerated in thickness. This sticks to the surface of the substrate, and the backward beating of the cilia drives the worm ahead.

1. Rate of Gliding Movement.—There is no very marked difference in the rate of the gliding movement in case of the species of *Planaria* studied. On the whole, specimens of *P. dorotocephala* move more rapidly than do those of the other two species, but there are large individual differences in this matter. Active specimens of *Dendrocœlum*, sp., move much faster than any other planarians I have observed. Large specimens of this form will sometimes glide along with simply amazing rapidity, not showing the slightest tremor of the surface of the body.

As to the absolute rate of the crawling, some statistics

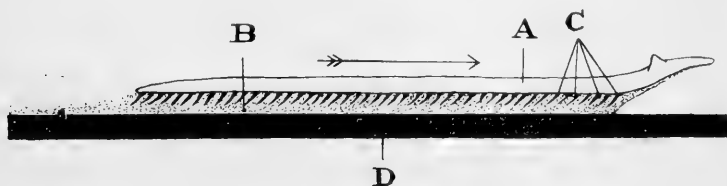


FIG. 4.—Diagram to show the mechanism of the gliding movement. A represents a specimen of *Planaria* seen from the side; B, the layer of mucus secreted by the animal. (This layer is represented as greatly exaggerated in thickness in proportion to the animal.) C, cilia. D, the substrate. The arrow indicates the direction in which the organism is moving. For further explanation see text.

have been collected and will be presented. The statistics were obtained in the following way:—A paper was ruled into centimetre squares; over this was placed a flat Petri dish containing the worm to be tested. Normal active specimens of *P. maculata* were used, and nothing was put into the dish but fresh clean water. The experiments were performed at night, and the source of illumination was a 16-candle power electric light enclosed within a ground glass globe. This lamp was 35 cm. above and 35 cm. distant in a horizontal direction from the centre of the dish, so that the light struck the animal at an angle of approximately 45° on its dorsal surface. The worm was allowed to get into an even,

normal glide, and then to come around, as it usually would in a short time, so that it was headed in the direction of the light. Then the time which it took the worm to glide three centimetres was taken by means of a stop-watch. If the animal started crawling, or abruptly changed its direction, the trial was ruled out. The average rate in millimetres per second determined in this way from twenty trials on two individuals is 1.34. This rate is considerably higher than those obtained by Parker and Burnett (:00) for *P. gonoccephala*. In that form they found a rate of 1.04 mm. per second in the case of individuals moving toward a horizontal light; 1.12 mm. per second when movement was away from a horizontal light, and 1.08 mm. per second when the animals were moving under a vertical light. There seems to be a well-marked correlation between the size of individual and the rate of gliding, as would be expected on general grounds, and is apparent from merely qualitative observations on the movement. One of the specimens from which observations were taken was 11 mm. long when extended, and its rate of gliding was 1.48 mm. per second; while the other specimen, which was only 6 mm. long when extended, showed a correspondingly slower rate of 1.23 mm. per second. The statistics are, of course, very meagre, and are not offered for any other purpose than to give a concrete idea of the approximate rate of the gliding movement. A thorough quantitative study of this matter of the rate of movement in planarians and other related organisms, and of the effect of different agents on the rate, would, I believe, be very interesting, and might lead to valuable results. I hope to be able to make such a study at some future time.

Lehnert (loc. cit., p. 17) gives a table of the rate of movement (presumably in the case of the fresh-water forms the rate of the gliding movement) of several species of flat-worms. He gives no account of how the data were collected, but his values may be inserted here for the sake of comparison. His rates for *Bipalium kewense* and *B. kewense viridis* are—Usual rate, 1 to 1.33 mm. per second; occasional rate,

1.83 mm. per second. This agrees very closely with the rate for *P. maculata* given above (1.34). His rates for *Geodesmus bilineatus* and *Dendrocœlum lacteum* are considerably slower (0.5—0.66 mm. per second and 0.75—1.33 mm. per second respectively). *Polycelis tenuis* (1.66—1.83 mm. per second), *Planaria polychroa* (2.16—2.5 mm. per second, exceptionally 3.33 mm. per second), and *Mesostomum tetragonum* (2.66 mm. per second) show a markedly faster rate than the forms I have studied.

Regarding the effect of different agencies on the rate of the gliding movement no special study has been made, and I can only report a few incidental observations. Such a study should be made by exact quantitative methods, and this I have not had the opportunity to do. What the effect of light on the rate is, it seems to me, impossible to say with entire certainty. Cole and myself¹ have found that light of great intensity (that obtained from a projection lantern with an electric arc as its source of illumination) causes a definite increase in the rate of gliding, but this increase has not been measured. The results of Parker and Burnett do not help us to answer this question of the effect of the intensity of light on gliding, as they are concerned only with the direction of its rays. The well-known phenomenon of “*Unterschiedsempfindlichkeit*” for light which *Planaria* shows (Loeb, '93, et al.) would indicate that increased light causes increased rapidity of movement. The electric current causes a very marked diminution in the rate of gliding in the weakest intensities which affect the organism at all. The effects of chemicals on the rate of gliding are not altogether uniform. Solutions of all chemicals tried with this point in view, when above a certain strength, caused a marked diminution in the rate of the gliding, or else an entire inhibition of it, and the substitution of some other form of movement. The action of weak solutions varied with the different substances. Very weak acids slightly increased the rate. Weak sugar solutions had no observable effect so far as rate of movement was con-

¹ Unpublished observations.

cerned. Copper sulphate causes an entire inhibition of the gliding movement in moderately weak solutions, even when these are not immediately fatal. Weak mechanical stimuli applied at the posterior end of the body cause a slight increase in the rate of gliding, but this is not marked, as any decided stimulus in this region of the body causes the crawling motion to supervene.

It is probable that the various agents affect the rate merely by causing changes in the general tonus of the animal. There is much evidence to support the view that the rate of gliding of an individual is a direct function of its tonic condition. Thus in the resting condition, which is characterised by a general lowering of the tonus, as will be brought out later, there is little, if any, ciliary movement. Again, after operations which result in lowering the tonus, the gliding is very slow or entirely absent.

2. Direction.—The direction of the gliding is, so far as my observations go, always forward. I have never been able to make the animal glide backward. This is in agreement with the finding of Child (:01) in the case of *Stichostemma*. It, of course, indicates that the effective beat of the cilia cannot be reversed. In the case of the planarians on which this work was principally done, a lateral change of direction of movement is not brought about by the stronger beating of the cilia on one side. In other words, when the animal turns to one side it does so by a muscular bending of the body in that direction, and not by ciliary action. In an undetermined species of triclad, however, I found that the most usual method of turning towards one side was by the stronger beating of the cilia on the opposite side of the body. As an individual was gliding along the bottom of the aquarium dish it would swerve off at an angle to its former course without bending its body in the slightest observable degree.

b. Crawling Movement.—The second form of locomotor activity, the crawling movement, is distinctly a muscular movement. It takes place only when the animal

has been stimulated in certain ways, and is of much less frequent occurrence than the gliding.

The crawling is always induced when the posterior end of the body is strongly stimulated. The characteristics of the movement are as follows:—If the posterior end of a worm which is gliding smoothly along is touched with a needle the posterior half of the body immediately contracts longitudinally; an instant later the anterior end stretches out far in front and fastens to the substrate. Then there is a longitudinal contraction which begins just back of the head and runs posteriorly. This, of course, at once draws forward the posterior part of the body, and as this comes forward and gets a hold on the surface on which the animal is crawling, the anterior end is again extended far in front and attached to the substrate. This process is repeated until the animal settles down into the regular glide again. It consists essentially in a stretching out of the head followed by a pulling of the body forward by an active muscular contraction. When the animal is very strongly stimulated the portion of the body posterior to the pharynx usually takes no part in the crawling after the first general contraction. In fact, the posterior half of the body may even be held slightly raised off the bottom, while the region between the head and the origin of the pharynx is actively expanding and contracting and sending the body ahead. These strong expansions and contractions of the anterior end which make up the crawling movement may follow each other in rapid succession as described above, or there may be a considerable interval between one contraction and the next. In this interval the body as a whole keeps moving ahead as a result of the ciliary action; that is, the gliding movement continues during the crawling, so that the latter may be regarded as an additional movement for the purpose of advancing the animal faster than the gliding alone can do it. The crawling may take place, however, with the ciliary beat entirely stopped.

The duration of the crawling movement after it is induced

is usually rather short. A single strong stimulus at the posterior end of the body—such, for example, as is given by running a needle through the body—will not usually cause more than three of the strong contractions of the crawling movement, and then the animal will relapse into the usual glide. The limits in this matter I found to be from a single contraction and expansion as a minimum up to six or seven as a maximum. This is, of course, in response to a single stimulus only. By repeating the stimuli the animal may be made to continue the crawling indefinitely.

The effective rate of this form of progression is faster than that of the gliding movement. The crawling rate of one of the worms used for the measurement of the rate of gliding (the specimen 11 mm. long) was measured in the same way as was the gliding rate. The worm was stimulated with a needle at its posterior end just enough to keep it crawling, i. e. prevent it from settling into the regular glide. The average rate of the crawling was found to be 1.66 mm. per second. Merely qualitative observation shows that the worm gets along somewhat faster in the crawling than in the gliding.

1. Direction.—The crawling may take place so as to carry the animal either forward or backward. The backward crawling is induced by very strong stimulation of the anterior region of the body. It does not always occur even after such stimulation, there being apparently some individual differences among the specimens in this respect. One factor which will call forth persistent backward crawling is partial desiccation. If the dorsal surface of the organism is allowed to dry, it will attempt to crawl backward violently. The mechanism of the backward crawling is just the reverse of that which obtains when the animal moves forward. The posterior end is extended and fastened to the bottom; then a wave of contraction, starting in this case from the posterior end, draws the remainder of the animal backwards, and then the posterior end is again extended. The backward crawling is usually induced when the worm is excessively stimu-

lated or injured at the anterior end. This movement almost always occurs when the head is cut off, and may usually be induced in such decapitated specimens for a considerable period after the operation by stimulating the anterior end. The backward crawling is not so rapid as the same movement forward. The reason for this appears to be that the posterior end is unable to take so firm a hold upon the bottom as does the anterior end. The backward crawling is usually not very long continued, the animal soon coming to rest. The inability of the animal to glide in a backward direction should, of course, be noted in this connection. Strong chemical stimulation of the anterior end will cause the backward movement to appear in some cases. Light, so far as I have observed, will not, nor will the electric current. There is considerable variation as to the appearance of this backward crawling. Some individuals cannot be induced to do it at all, or only in a very slight degree, while others will crawl backward for considerable distances after injury to the anterior end. It appears to be a complex of reflexes which under normal circumstances is inhibited, and only appears in any pronounced way under comparatively rare conditions. It is not, as might be expected, a method ordinarily used by the organism to get out of danger. This is one of the cases quite frequently met where an organism has among its available assets, so to speak, a reaction which is well adapted to a certain end, but of which use is not made at all, or but very little.

2. Stimuli which induce Crawling.—It may be said in general that almost any strong stimulus applied to the posterior portion of the organism causes the forward crawling movement to appear. Mechanical and strong chemical stimuli applied in this region will do this. Light, either of ordinary intensity, or of such high intensity as that from an arc light, so far as I have observed, will not cause the crawling movement. The electric current does cause it, but greatly diminishes the rate. Any operative treatment—as, for example, cutting the body in two in the middle—almost in-

variably causes the portion in front of the cut to advance by the crawling movement, and, as has been mentioned in the preceding section, at the same time frequently causes the posterior piece to crawl backward. There is no reason to suppose that the operative procedure acts in this respect in any other way than merely as a strong mechanical stimulus applied at the point of the cut. Other stimuli which induce the backward crawling have been taken up in the preceding section.

c. Movement on the Surface Film.—Motion on the surface film is practically confined to the gliding movement. This gliding is slower in rate than that on the bottom, largely on account of the greater flexibility of the surface on which the animal is moving. While the mechanism of the movement is the same in the two cases, the surface film is elastic, and does not give so firm a basis as does a solid body. The effect of this elasticity of the film is very well seen when the animal attempts to change its course and turn to one side. The film offers little resistance to the posterior end, so that this cannot easily serve as a fixed point for the anterior part to turn about. Furthermore, in case the anterior end is left in contact with the film when the turn is attempted, as is usually the case, there is almost as much resistance against this turning of the anterior part as there is resistance to hold the posterior end fixed as a pivot support. The consequence is that the worm is unable to change its direction of movement quickly when on the film, and it has to go through a succession of muscular twists and jerks towards one side before the result is attained. I have not been able to induce well co-ordinated crawling movements in a worm while on the surface film. The preliminary contraction of the posterior part of the body occurs when that region is stimulated, but the subsequent stretching out of the head and drawing up of the body does not usually follow. I have tried stimulating both the exposed ventral surface of the animal and the dorsal surface from below, but neither method is effective. The reason for this is probably to be found again in the elasticity

of the film. The anterior end is unable to get any firm attachment so that the rest of the body may be drawn forward. Furthermore, similar resistance is offered to the stretching of the head forward as to the turning of it towards one side.

When the animal is gliding on the surface film the same raising of the head (with reference to the worm, of course: in this case, a lowering with reference to the centre of the earth) and waving it about in the water occurs as under normal circumstances. In some cases two thirds or three fourths of the whole body will be thus raised and waved about, extending itself to its utmost capacity, and apparently seeking some solid body on which to attach itself. In these cases only a small portion of the very posterior end of the body will be left in contact with the film to support the whole.

On coming to the side of the dish when gliding on the surface film, the worm almost invariably leaves the film and turns down the side of the dish. The reaction which is the cause of this and of the organisms passing from the side of the dish on to the film will be brought out later.

d. Relation of Movements of Triclad s to those of other Forms.—In respect to their movements, the triclads studied occupy a somewhat intermediate position between certain other groups. The rhabdocœles are in general characterised by free movements in the water, brought about by cilia covering the whole body. Their movement in general features resembles that of the holotrichous Infusoria. A type showing well this class of movement among the Turbellaria is *Stenostoma leucops*. The movement is not at all or very little dependent upon muscular activity. On the other hand, the movement of many of the polyclads is characteristically muscular. An example of this is found in the case of *Leptoplana tremellaris*, where the movement is largely effected by the rhythmical beating of the margins (cf. Lang, '84). In fact, this form of movement has become so well developed in these animals

that we may think of the margins of the body as special locomotor organs. Ciliary action plays little if any part in the movement of such a form. It is to be noted, however, that both the rhabdocœles and the polyclads are capable of performing true swimming movements, i. e. movements free in the water without contact with any solid body. In the fresh-water triclads, especially of the genera *Planaria* and *Dendrocoelum*, the cilia have become much diminished in comparison with the rhabdocœles, and are restricted to a portion of the ventral surface only. Consequently they are not numerous and strong enough to support and move the disproportionately heavier body freely through the water. The movement of the cilia merely serves in these forms to propel the body while insufficient to support its weight. Consequently we find the principal form of movement to be a gliding over the surfaces of solid bodies.¹ On the other hand, the fresh-water triclads have not attained the high development of muscular locomotion which the polyclads have. There is a purely muscular movement in their case, but it is not by far the most important form of locomotion, and is not so highly developed as is that of the polyclads. Evidently, then, the fresh-water triclads seem to form a transitional stage in respect to locomotor phenomena between the rhabdocœles on the one hand, where purely ciliary locomotion obtains, and the polyclads on the other hand, where we find the locomotion largely if not entirely muscular. Whether this has any phylogenetic significance is not certain.

The land planarians occupy a position very similar to that of the fresh-water forms so far as their movements are

¹ I do not wish to imply, in this discussion of the different forms of movement as related to the number and distribution of the cilia, any belief that structure gave rise to function or function to structure. I wish merely to point out the evident correlation which exists in the matter. It seems to me most probable that structure and function changed together; but in this, as in many other similar cases, positive evidence is lacking, and consequently attempts to settle the phylogenetic development of the phenomena would appear to be fruitless.

concerned. There is, however, a noteworthy difference between the two groups. In the movements of the land planarians the muscular factors (rhythmical wave motion of the ventral surface, and snake-like movements of the whole body) are more important relatively to the ciliary component than in the fresh-water forms. In these land planarians there is evidently the beginning of the characteristic rhythmical wave motion of the part of the body in contact with the substrate, which reaches its highest development in the case of the Mollusca.

II. Non-Locomotor Movements.¹

Under non-locomotor movements will be included the phenomena of contraction, expansion, "feeling movements," movements of the pharynx, etc. The purpose of discussing these phenomena, which are not immediately included in the general standpoint, is to give an account of their mechanism which may be referred to in succeeding portions of the paper. These movements are the physiological foundations on which the locomotor movements and the reactions are based, and it is necessary to determine their mechanism in order to bring the analysis of the behaviour to completion.

a. Contraction of the Body.—By the term "contraction of the body," when applied to forms like the flat-worm, is usually meant the shortening of the body lengthwise. In the flat-worm this movement is brought about by the contraction of the longitudinal muscle-fibres. It may involve the whole body or only a portion of it. Most frequently only a part of the body contracts longitudinally after stimulation; thus, if the anterior end is rather strongly stimulated in the middle line, the resulting contraction will usually involve only the anterior third of the body. In this longitudinal contrac-

¹ In discussing the musculature I have used throughout the nomenclature of Jijima (loc. cit.), in whose paper a very full description of this system will be found. I have identified in sections of *P. maculata* the following groups of muscle-fibres:—(*a*) outer longitudinal, (*b*) circular, (*c*) oblique (?), (*d*) inner longitudinal, (*e*) dorso-ventral, (*f*) transverse.

tion all the sets of muscle-fibres other than the longitudinal must be relaxed completely, because as the animal shortens it grows broader and thicker, which would be impossible if the ring, or transverse or dorso-ventral musculature, also contracted. The longitudinal musculature is apparently better developed and more effective on the ventral side of the body than on the dorsal, because after very strong stimulation of the anterior end there is a well-marked tendency for the middle portion of the body to be raised and the head somewhat curled in under it. This relation is shown diagrammatically in Fig. 5. This curling under of the head does not appear to be a specific reaction, but, on the contrary, merely an expression of the fact that the ventral musculature is capable of shortening its side of the body more in maximal contraction than the dorsal side. Jijima (*loc. cit.*, p. 378)

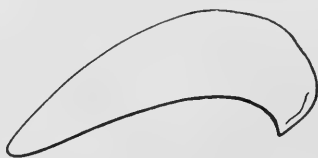


FIG. 5.—Diagram showing the appearance in side view of a maximally contracted planarian.

finds, from a histological study of the musculature, that the bundles of fibres in the main longitudinal muscle layer are thicker on the ventral than on the dorsal side.

b. Extension of the Body.—The mechanisms by which extension of a soft-bodied animal is brought about are probably very different in different groups. In the case of the flat-worm extension is produced by the contraction of the circular muscular layers surrounding the body, and of the transverse and dorso-ventral systems of musculature. Probably also the oblique musculature, when present, assists, by its contraction, in the extension of the body. The mechanical necessity for extension of the body, after contraction of these muscles, is readily apparent. If the body, for simplicity's sake, be considered a cylinder, contraction of circular

muscles must cause it to lengthen, while with the form of body which really exists in the flat-worm, the contraction of the well-developed dorso-ventral fibres must bring about the flattening seen in the fully extended gliding animal.

Probably by far the most important sets of muscle-fibres for producing the general extension are the dorso-ventral and circular. It is to be noted that contractions of any of the sets of fibres may take place in localised regions, producing extensions or contractions of that region, according to the set affected. The sensory, or "feeling" movements of the head are brought about in this way.

The extension, and probably also in large part the extrusion of the pharynx is brought about by contraction of its well-developed circular musculature.

In the case of the marine mollusc, *Aplysia limacina*, Jordan (: 01, pp. 11—15) has recently shown that extension is brought about in an entirely different manner. It results from passage of fluid from vesicles in the skin into the spaces in the body parenchyma when the body muscles are relaxed. When the animal contracts this fluid (blood) is pressed out into the vesicles, which become very much extended and swollen; then, when the muscles are relaxed, the elasticity of the walls of the vesicles forces the fluid back into the body, and thus causes its extension. As a result of this method of expansion it is possible to kill the animal fully extended by the use of such poisons as cocaine.

It is not unusual to consider the fully extended condition of such an organism as a flat-worm as one of approximate relaxation. Instead of this, it is, in fact, a condition in which a certain part of the musculature is in a state of well-marked tonic contraction. This furnishes a reason for the fact that it is impossible to kill these animals in a completely extended condition by the use of poisons which tend to produce a relaxation of the muscles. Under these circumstances the animals take on the typical relaxed form, which is quite different from that of extension.

c. Rest.—Inasmuch as a very large, if not the larger

portion of the life of a planarian is spent in a condition of rest, it will be well to discuss this matter; and it may, perhaps, best be taken up under the general heading of "activities," although really the opposite of activity.

The appearance of the worm when resting is, as has already been mentioned, quite different from its appearance in the active condition. The body is shorter, wider, and thicker. The ordinary contour of the head is almost entirely lost, and in place of the sharply pointed anterior end of a form like *P. dorotocephala*, the end is evenly rounded. The auricles disappear almost entirely, and their position is indicated only by the difference in the pigmentation at that part of the dorsal surface. The lateral edges of the body frequently have

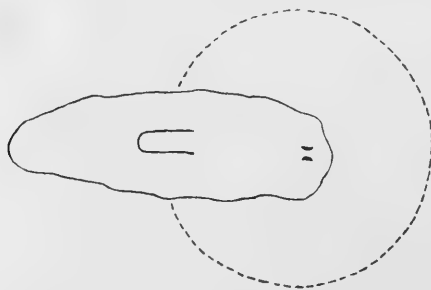


FIG. 6.—Diagram showing the typical appearance of a resting planarian. The dotted line bounds approximately the area covered in the "testing movements" which precede the coming to rest (cf. text).

a wavy line instead of the straight one of the active condition. The anterior end of the body is in contact with the bottom, and not raised as in movement. The general appearance of a resting planarian is shown in Fig. 6.

The coming to rest of a gliding animal is usually done in a very characteristic way. First, the animal glides more and more slowly for some distance before reaching the point at which it will finally stop. The distance before reaching the stopping place in which the worm glides appreciably slower is not, however, in most cases very considerable—usually not more than two or three times its own length. It is to be noted that this slower gliding which precedes the coming to

rest is not in form or rate distinguishable from the other slow gliding motion of the worm which is not followed by rest. In other words, a specimen may glide slowly for a long time without stopping, so that one cannot prophesy with certainty from the rate of movement whether the specimen is soon coming to rest or not. The coming to rest is practically always preceded by a period of slower gliding, but all slow gliding is not immediately followed by rest. After a brief period of this slower gliding the worm suddenly stops, and the posterior half of the body remains fixed in precisely the same position. The anterior half of the body is slowly moved about over the bottom from side to side, the head being touched frequently to the bottom or any other solid object in the neighbourhood. The anterior part in this "feeling" movement moves about the posterior part as a fixed point, the latter very rarely changing its position after it has once stopped. The thoroughness of this "testing" of the surroundings by the sensory anterior end varies much in different cases, but in practically all cases one can see some indication of it. I have in some instances seen it done very thoroughly, so that the whole surroundings within a radius of 3 mm. were gone over. Finally, when this is done the animal comes to complete rest, and assumes the typical relaxed condition shown in Fig. 6. The apparent significance of the "testing" movements at the time of stopping is that it is a piece of protective behaviour. The worm examines the surroundings before coming to rest, to see if there is anything dangerous (either of a solid nature or a harmful chemical) in the immediate neighbourhood. Whether or not this explanation is the true one, and further, whether natural selection developed this reaction for protective purposes, seems to me to be very doubtful, for reasons brought out in another place (cf. pp. 542 and 543). In some cases I have seen the worms come to rest by simply stopping without any appreciable trace of the "feeling" movements, but this is not the usual procedure. In coming to rest in one of the collections already mentioned, the "feeling" movements are usually very well marked.

There is a well-marked tendency for the planarians studied to come to rest in such a way that the long axis of the body forms a right angle, or nearly a right angle, with the lines of the force of gravitation. The cases in which the organisms come to rest with the long axis forming an angle of less than thirty degrees with the line of gravitation are rather few. Of course, when they come to rest on the bottom, the angle formed is approximately ninety degrees. A large number of observations on individuals which came to rest on the sides of dishes of various shapes have given the general result stated above. There are, of course, exceptions, but there is,

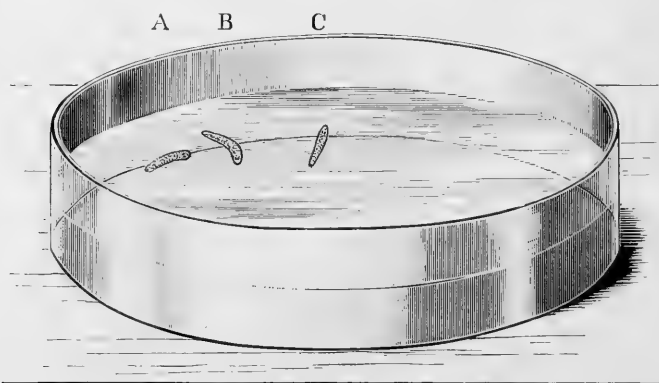


FIG. 7.—Diagram showing the positions taken by planarians coming to rest in a dish (see text).

after making due allowance for these, a tendency to a horizontal position as the position of rest. This is the only behaviour of the organism which bears any resemblance to a geotactic reaction. Another tendency, less marked than the former, is for the animals to come to rest in the angle formed by the sides and bottom of the dish. Not only do specimens come to rest lying directly in the angle, as shown at A in fig. 7, but also, and more frequently, they lie in such a position that a part of the body is on the side of the dish and a part on the bottom, as shown at B, fig. 7. In this position the animal usually lies obliquely rather than at right

angles to the line of the junction of the side and bottom of the dish. The animals usually come to rest in this position after they have been gliding on the side of the dish. When they come to rest from movement on the bottom of the dish, a position frequently taken is that shown in C, fig. 7, where only the very anterior end is in contact with the side. This coming to rest in the angle of a dish is apparently a reaction which agrees with those usually called thigmotactic reactions. But it is not, as has been stated by several writers, due to a tendency to get more of the body in contact with something solid, than is in such contact under usual conditions; for in the case of an organism like a flatworm, it is impossible for any more of the surface of the body to be in contact with a solid when it is bent, as shown in Fig. 8, A, than when it is flat, as in B. There is the same amount of surface in contact in either case. The ventral surface of the flat-worm is

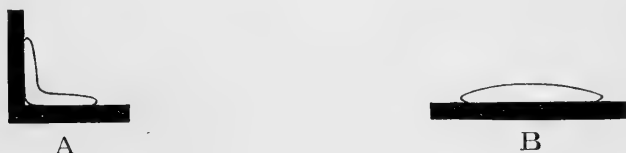


FIG. 8.—Diagrammatic cross-section of a planarian at rest—A, in an angle; and B, on a plane surface.

strongly positively thigmotactic under all circumstances, and the dorsal surface negatively thigmotactic, but this does not help us understand why the animal comes to rest frequently in angles. This behaviour of the flat-worm in dishes is due to the same sort of reaction as that which causes them to come to rest on unevennesses on rocks, and also causes the same phenomenon in a more marked degree in the case of *Littorina*, as recently described by Mitsukuri (:01). The common factor in the reaction in all cases is that different parts of the body are brought into such positions that they form unusual angles with each other. Since this phenomenon is distinctly different from any embraced by the term thigmotaxis as used in its true sense, it seems desirable that it be

given a specific name. I would propose for this reaction the term *goniotaxis*.¹

When a flat-worm starts from a resting condition the nature of the movement, i. e. whether gliding or crawling, depends in large measure on the intensity of the stimulus which starts it. If the resting animal is rather strongly stimulated it will start at once into a crawling movement, which changes to gliding after three or four, or fewer, contractions, provided the stimulus is not again renewed. It is possible by the use of a very weak stimulus to start the resting animal off at once into the gliding movement, or with only the faintest indication of a single crawling contraction. When the animal starts spontaneously into movement it usually begins at once with the glide. When starting spontaneously the glide is usually preceded by some of the "feeling" movements of the head end, such as precede the coming to rest. The purpose of these is evidently the same as in the former case. Any sort of strong stimulus will start the resting animal into movement.

The physiological condition of the resting animal is, as has already been mentioned, one of relaxation. All of the muscular systems of the body are in an apparently completely relaxed condition. This is evidenced by the form of the resting animal, which differs from that of one in movement, in being shorter, wider, and thicker, and in not showing such features as the auricles, or the pointed tip of the head. This relaxed condition is evidently one of lowered tonus, as may be determined by simple observation. Stimuli of an intensity which would cause a marked reaction in an individual in an active condition, will produce no effect on a resting animal. This point has been tested with a variety of stimuli, including mechanical, chemical, food, etc., and the markedly lower tonus of the resting animal is very evident. The reactions which are produced, provided the stimulus is made strong enough to be just effective, are weak. Of course, if the stimulus is above a certain strength, or is con-

¹ From *γωνία* = angle.

tinued for some time, the animal will become generally stirred up and glide or crawl away. This condition of lowered tonus in the resting animal reminds one of the conditions found in sleep in the higher animals. There, as in this case, the general sensory and muscular tonus is greatly reduced, and there seems to be no good reason why the resting condition of these lower organisms may not be considered and called "sleep." The two things appear to be fundamentally the same physiologically, and would appear to serve the same purpose. Furthermore, there is no apparent reason why the lower organisms should not have as great a need as the higher for periods of rest or sleep; during which the anabolic processes are in considerable excess over the katabolic. The fact that some lower organisms are so balanced physiologically that they apparently do not require such periods of rest is not conclusive evidence that other low organisms must be similarly balanced. So far as is known to the writer, there has been comparatively little attention paid to the physiological condition of lower organisms during different phases of their activities. An animal which is not moving is loosely said to be in a "resting condition," when in many instances, as in *Clepsine*, the quiet animal is in a condition of heightened rather than lowered tonus (cf. Whitman, loc. cit.).

As was noted in the section on "Natural History," the periods of activity of *Planaria* are separated by periods of rest of greater or less length. The time spent in the resting condition, at least during the daytime, is considerably greater, on the average, than that spent in movement. Probably, however, this is reversed during the night, when the activity is greater than during the day. This periodicity in the activity is just what would be expected if there is a necessity for rest at intervals as in the higher animals.

The causes which immediately induce the coming to rest may now be considered. The principal cause, as has been indicated above, is that the animal becomes fatigued by

movement, and its general tonus becomes lower and lower. As a result of this it must remain relaxed for a certain time in order that recovery may take place. When in the course of the activity of the animal its general tonus gets below a certain point it stops, the actual process of coming to rest being a more or less gradual one. A strong piece of evidence in favour of this view is the fact already given in the section on "Natural History," namely, that if the animal is stirred up and made to start moving again immediately after coming to rest each time, it will be found that the periods of activity become progressively shorter. Furthermore, when the general physiological condition of the organisms is weakened by keeping them for a time in the laboratory, it is found that the periods of rest become progressively longer in proportion to the periods of activity. The general "predisposing condition" to the coming to rest is then probably a lower tonus due to fatigue. The immediate causes determining the exact place chosen are of three sorts. First, and probably most important of these, is the intensity of the light. It is well known that planarians tend to come to rest in regions of comparatively low light intensity, the reaction having been first noted by Loeb ('93), and called by him "Unterschiedsempfindlichkeit." This factor seems to be the most important of any in determining the region in which the animals come to rest, both under experimental conditions and in the natural habitat. In aquarium dishes placed close to a window, and containing considerable plant material, the worms will be found resting practically always in the half of the dish away from the window. The largest number of individuals will be entangled in the plant material, and usually for the most part invisible; while of those specimens resting on the sides and bottom of the dish the greatest number will be found in such places that there are heavy masses of plant material between them and the window. A few will come to rest far around on the sides of the dish where the glass itself cuts off some of the light. This last position has been mentioned by

Loeb as the one most frequently taken by planarians in a dish containing only water. This behaviour towards light is not, however, an absolutely precise reaction. Many times during experiments I have seen specimens come to rest in the very lightest parts of the dish and remain there; but in general this reaction will cause most of the animals to gather in shaded areas. It is probably the principal factor in causing the animals to take positions beneath stones in their natural habitat.

The second immediate cause in determining where the animals shall come to rest is the goniotaxis mentioned above. If an animal in the proper physiological condition of reduced tonus comes to an unevenness in the surface on which it is moving, it will in most cases come to rest there. This, again, is not a very precise reaction; not sufficiently so as to make it possible to predict beforehand where any given individual will stop. In this case, just as in the case of light, much depends on the animal's physiological condition, and when in the proper condition they may come to rest on a perfectly smooth surface. Thus in a dish individuals will always be found at rest on the smooth sides and bottom, yet there is a distinctly marked tendency, when the animals are put under experimental conditions and closely observed, for them to come to rest in the angle of the dish. This reaction probably also plays a considerable part in the habit of coming to rest among the branches and leaves of the plant material. In the natural habitat it is undoubtedly the factor which causes them to take positions on the uneven parts of stones. It may be that the immediate cause of the stopping in this case is the increased resistance to movement afforded by the unevenness of the surface. This, acting on an animal in a fatigued condition, might give the necessary stimulus for the stopping.

The third factor in determining where the animals shall come to rest is one about which I am doubtful. There seems to be some evidence, from the behaviour of the animals themselves, that in the formation of the groups or collections

previously mentioned (pp. 533, 534) there is a sort of chemokinesis. That is to say, the presence of some chemical substance in the water causes the animals to stop. The evidence for this factor will be taken up with the discussion of the formation of collections. It probably does not play any part in determining where a single individual shall come to rest outside of a collection.

It must be emphasised that all of these three factors are secondary in importance as compared with the physiological condition of the animal, which may be said to prepare it for the resting state. An active animal, in which the tonus is at or near the maximum, will pass through regions of low illumination, uneven surface, or collections of other individuals without stopping. Only when the animal is in the right general condition do these factors come in to determine the precise point where the stop shall be made.

1. Formation of Collections.—Since the formation of collections is dependent on the animals coming to rest in a certain area, it may properly be taken up in this section. The collections are fairly well-defined groups of from six or eight up to twenty or more individuals. The general appearance of such a group is shown in Fig. 1. The individuals composing it have no definite orientation, but are scattered about with the anterior ends directed in whatever way they happened to be pointed when the individuals stopped. The distance separating the individuals varies much in different cases. In some cases it may be as much as a half-centimetre, or again may be the width of an individual worm or less. This formation of collections of this sort might be considered the result of a "social instinct" by animal psychologists of the Binet school. Actually, it appears to be due to two simple reactions taken in conjunction with the general physiological condition of the individuals composing it. The first of these reactions is that to light. That is to say, when individuals come to a comparatively restricted area of a certain degree of illumination, if they are in a certain condition of reduced tonus, they stop. Those which are

very active pass on through the region, but necessarily in course of some time several individuals will have stopped, and a group will have been begun. When once started another reaction apparently enters to assist in enlarging it. This reaction appears to be due to some chemical substance, and belongs to the class of reactions which Engelmann has suggested should be called "kinetic," in this case chemokinesis. It would appear that planarians excrete or secrete some chemical substance towards which they are themselves positively chemotactic, and which also causes them to come to rest. When several individuals remain quiet in a small area this substance, of course, accumulates and affects other individuals passing. That some such a substance is separated from the bodies of the animals is evidenced by two phenomena. First, in the case of the food reaction, which will be taken up in detail later, it is found that after one or two individuals have attached themselves to a piece of food material and begun feeding the mass of food and planarians is a much more effective stimulus to positive chemotaxis than is the same food substance alone, even though it may have remained in the water a greater length of time. The "zone of influence" (*vide infra*, p. 626) of the food and feeding individuals together is much wider than that of the food alone. Specimens are affected at a greater distance from the food and react more sharply. As a result of this, dense aggregations of planarians will be formed in a comparatively short time after the first two or three individuals have found a bit of food. As there is no reason to suppose that the action of the food itself is different in the two cases, we must conclude that the greater effectiveness of the food and feeding individuals is due to some chemical substance coming from the organisms themselves.

The second line of evidence for the existence of a reaction to a chemical in the formation of collections is found in the behaviour of specimens coming near a group of individuals resting on the bottom of a dish. When some distance away from the outer boundary of such a group a gliding animal

will frequently be seen to give a well-defined positive reaction, and turn towards the group. The reaction is of precisely the same character as that given by the organism to weak chemicals (to be described later), and the behaviour convinces one observing it that the specimen is stimulated by some chemical diffusing out from the group. After turning towards the group the specimen will glide into it and usually come to rest, in the manner which has been described above.

What the nature of the chemical substance present in the region about the groups is, I have not been able to discover. Neither rosolic acid nor methyl orange is discoloured by it. Whatever its nature, it must be in an extremely diluted state. This seems evident for two reasons: first, because it does not affect delicate indicators; and second, because it does not have any effect on active specimens of *Planaria*. A large number of experiments have been performed to test this latter point, but always with the same result. Unless the individuals were in the proper predisposing condition of lowered tonus, they would pass by or through groups of other individuals without giving any reaction.

Attempts to produce, artificially, collections of planarians in chemicals have been unsuccessful. I have tried various solutions (such as sugar, weak alkalies, etc.) to which the organisms showed a well marked positive chemotaxis when tested by other methods, but have not been able to get any formation of collections in them. The animals would give the positive reaction on coming to the edge of the diffusing chemical and pass into it, but would not come to rest. This failure to produce collections artificially is not surprising when one considers the number of conditions necessary for the production of the desired result. The organism must be in just the right physiological condition, the chemical must be of a certain concentration, and finally, it must be located in an area of a certain light intensity. It is practically almost or quite impossible to fulfil all these conditions at the same time in an experiment.

The coming to rest in the collection seems to be due simply to the direct effect of the chemical on the organism. There is no evidence that the animals are held in the group as a result of a negative reaction to the surrounding water, as is the case in the collections formed by the infusoria (cf. Jennings, '99, *b*). The method of formation of collections in chemicals in the case of the infusoria is as follows:—Specimens swimming about at random come to the edges of drops of chemicals purely by chance. If, for example, the chemical happens to be a weak acid, the specimens will pass into the drop without giving any reaction. When, however, they reach the opposite edge of the drop and attempt to pass from the chemical back into the water they are stimulated, and give their usual motor reaction. This turns them back into the drop, in which they are, as it were, “caught in a trap.” As a consequence of this method of reaction a very dense collection will be formed in a short time. With the flat-worm the case is very different in that an active individual frequently passes into and out of one of these collections without showing the faintest trace of a reaction on either side. The only way in which any stopping in the region is brought about by a chemical is by a chemokinetic reaction. The fundamental difference in the reactions of the two groups of organisms on which this difference in the matter of forming collections is based will be brought out in the section on the reactions to chemicals.

To sum up, the formation of collections of individuals seems to be due, in the first instance, to the tendency of the organisms to come to rest in areas of a certain degree of intensity of light, and in a lesser degree to a tendency to turn towards and come to rest in areas containing some substance secreted or excreted by the worms themselves. A prerequisite in the formation of collection, as in the coming to rest under any circumstances, is a proper physiological condition of reduced tonus.

There does not appear to be any special biological significance to this tendency of the animals to collect in groups.

The behaviour is not of any evident benefit to the organisms, as it is in the case of infusoria, where it is apparently closely connected with the obtaining of food. On the contrary, it seems to be, at least potentially, a harmful thing, because any accident or enemy would affect a number of individuals rather than a single one when they were so collected.

d. The Effect of Operations on Movement. — It may be well to put together in one place the results which were obtained with reference to the movements from animals which had been cut in various ways. From these we can form some idea of the relation of the nervous system of the planarian to its movements.¹

The immediate effect of any operation is that of a very strong mechanical stimulus applied to the same part of the

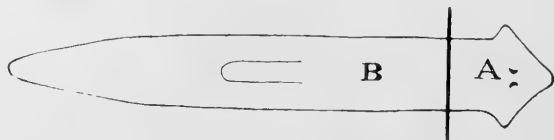


FIG. 9.—Operation diagram. The heavy straight line indicates the cut made. For results see text.

body, and the sort of movement resulting in each piece depends on the position of the cut. The details of this immediate effect will be described in connection with other mechanical stimuli. What concerns us here is the permanent after-effect of operations on the movements. We can best get at this matter by taking up some specific cases.

¹ All the operations were performed with a sharp scalpel, in most cases with the specimen in a dish of water. In some cases the worm was transferred to a drop of water on a soft board for the cutting, but in all cases where immediate observations were wanted, the operations were performed in the dishes used for the experiments. The only difficulty in performing operations on planarians arises from the fact that if the edge of the knife is allowed to rest on the surface of the body for even a very short time before the cut is made, it will become covered with the sticky slime from the animal, and then any clean cut is impossible. The edge will slip off the back of the worm without penetrating.

If a planarian is cut squarely across the body in the region a short distance behind the head, as indicated in Fig. 9, the anterior piece will continue to move after the operation at approximately the same rate as the whole animal did before. After the immediate effect of the operation is past the glide is its ordinary movement, and it will go about the dish and behave in general like a whole individual. At the outstart its periods of activity and rest are distributed about as in a normal individual, or, in other words, its power of spontaneous movement is not impaired, at least for a time. On the other hand, the posterior piece comparatively soon comes to rest after the operation. Its gliding movement is slower, and the periods of rest become longer and longer in comparison with the periods of activity. Its power of spontaneous movement becomes very greatly diminished within a comparatively short time after the operation, and it remains



FIG. 10.—Diagrammatic side view of a decapitated specimen performing the gliding movement.

in the relaxed resting condition during the greater part of the time spent in the process of regeneration. When this posterior piece does glide about soon after the operation its anterior end is usually raised off the bottom considerably higher than is the head of a normal flat-worm under similar circumstances. This is shown in Fig. 10. There are no "feeling" movements of the anterior end of such a piece, but instead this end is held very stiffly in the raised position.

If, instead of making the cut so close behind the head, it is made back in the middle region of the body, the anterior piece behaves as before, i. e. like the normal animal. The posterior piece, however, moves slower than did the corresponding piece in the previous experiment, and it comes to rest sooner after the operation, and remains quiet longer.

In the same way cuts may be made nearer and nearer the posterior end; the posterior piece will move more and more

slowly, and come to rest sooner. At the same time the anterior pieces will appear more and more like the normal. In both sets of pieces the crawling movement may be induced by proper stimulation of the posterior ends.

Oblique transverse cuts produce the same results as do

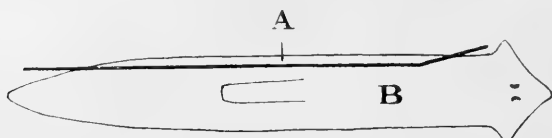


FIG. 11.—Operation diagram. Heavy lines indicate the cuts made. For results see text.

direct ones. The same laws hold as to the movements of the pieces. In case a strip is cut from the side of the body, as shown in Fig. 11, the smaller piece A curls up, and does not make any further progressive movements, although it remains alive, and will eventually regenerate in most cases. The main part B contracts on the cut side, and hence becomes curved in that direction after the operation. It is able to move about, but at a somewhat slower gliding rate than normal, and in a path curved towards the cut side. In case a worm is slit down the middle line at the anterior end, as in Fig. 12, it is able to glide, but at a slower rate than normal. It performs the crawling movement in response to stimulation at the posterior end, and each half of the head performs feeling movements independently of the other half.

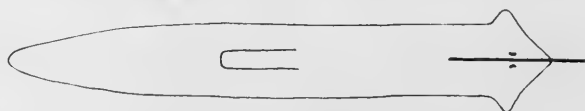


FIG. 12.—Operation diagram. The heavy line indicates the cut made. For results see text.

An individual slit up in the middle line from the posterior end, as in Fig. 13, glides at approximately the normal rate, provided the cut is not carried too far forward. If the cut extends into the head region the gliding becomes immediately slower. Such a specimen performs the crawling

movement upon stimulation of the posterior end of either piece, but in a peculiar way, which will be described later.

Putting all these results together, we see that there is a general tendency for animals on which operations have been performed to glide at a slower rate than normal. In some of the pieces this tendency is very slight, and frequently hardly noticeable. In others the movement is very much slower than normal. In all cases the periods of rest are longer during the time of regeneration than normally. This tendency for the animals to remain quiet during regeneration increases up to a certain point as regeneration proceeds. A piece of a planarian may be quite active for three or four hours after the operation, while during the following three or four days it will scarcely move at all. After the regeneration is practically complete the worm will begin to move about again approximately as it normally does. During the re-

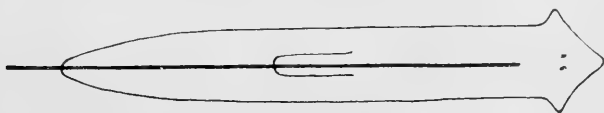


FIG. 13.—Operation diagram. The heavy line indicates the cut made.
For results see text.

generating process the anterior pieces, bearing an uninjured head, are much more inclined to move about than are the posterior parts. These latter usually remain entirely quiet during regeneration.

This behaviour of the posterior parts during regeneration appears to be distinctly purposive, and to belong to the class of phenomena called regulatory. The general tonus of these pieces is immediately lowered by the operation, and consequently they keep quiet. Yet at the same time the processes of morphallaxis and, in many cases, growth begin at once, and proceed very vigorously till the missing parts are restored. If we consider that the worm or part of a worm has at the beginning a certain sum-total of energy available for all activities, including movement, growth, morphallaxis, and all its other vital processes, then it would appear that

the performance of any single set of activities in excess must cause a corresponding diminution in other activities. This is exactly what we find to be the case with the regenerating planarian. While the processes concerned in regeneration are at their maximum activity, we get a decided reduction in the amount of movement. It would seem, then, that a large part of the energy which is ordinarily expended in movement is used after operation or injury in the processes of regeneration. As the regeneration nears completion more and more energy is available for, and used in movement. This would seem to be a sort of "energy regulation." The behaviour is evidently further beneficial in the case of the posterior pieces, because their anterior ends are very insensitive as compared with the head of the normal animal, and if they moved about they would certainly be more apt in the long run to get into difficulties than if they remained quiet.

It may be well in closing the section to point out the relation of the nervous system to the movements. Loeb (:00) has maintained that "if we divide a fresh-water planarian, for instance *Planaria torva*, transversely, the posterior half, that has no brain, crawls just as well as the oral half. Spontaneity in *Planaria torva* is, therefore, by no means a function of the brain." If by "crawl" in the first sentence we understand "glide" to be meant, the statement is not strictly accurate. The posterior pieces do not "move just as well as the oral" (anterior), but, as has already been brought out, more slowly. For a very short time after the operation the statement would in some cases be correct, but it certainly would not be twenty-four hours later, according to all the observations I have been able to make on the subject. As for the spontaneity of the movement, that also becomes very much lowered with the loss of the brain, as I have attempted to show above. The very much lessened activity of posterior pieces of planarians has been mentioned by Lillie (:01, pp. 132, 133).

From my own observations it seems clear that the principal

function of the brain of *Planaria* with reference to movements is to maintain the tonus of the ciliary system. That neither the crawling nor the gliding movements are specific functions of the central nervous system is evident, because both sorts of movement may take place after its removal. Yet all my observations tend to show that after injury to or loss of the brain the gliding movement becomes, almost immediately, markedly slower. This relation is especially well indicated by the experiments noted above on splitting the animal longitudinally from the anterior and the posterior ends. In the one case the gliding movement becomes at once distinctly slower, while in the other case there is only a slight difference in the rate, evidently conditioned by the fact that only comparatively few of the cilia can get a hold, so to speak, so that they can function. The force of the argument will be impressed if one glances at the relative size of the cuts in Figs. 12 and 13, and then remembers that the rate of gliding of the specimen figured in Fig. 13 is faster than that of the one in Fig. 12. With the co-ordination of movements, including the crawling, the central nervous system has very little to do in the case of *Planaria*. With regard to the spontaneity of movement it is difficult to decide in how far the brain functions. It is certain that regenerating anterior pieces show more spontaneous movement than do posterior pieces, yet the anterior pieces are behind the normal worm in this respect. The brain probably plays some part in the performance of normal spontaneous movements, but, as has been pointed out, in these operation experiments the whole matter is very definitely related to the regenerative process, and loss of substance plays nearly, if not quite as great a part as loss of nervous system.

Summarising, we may say that—1. For the performance of the crawling or gliding movements the brain is not specifically necessary. These movements are normally co-ordinated in the absence of the brain.

2. The maintenance of the tonus of the ciliary system (which produces the gliding movement) is a specific function

of the brain, and is, further, its most important function so far as movement is concerned.

3. The brain plays a certain part in the production of spontaneous movements.

F. REACTIONS TO STIMULI.

I. Reactions to Mechanical Stimuli.

Since the reactions which are given by *Planaria* to mechanical stimuli are in a sense the foundation on which the reactions to other stimuli are based, it may be well to consider them first. After thoroughly working out the reactions to mechanical stimuli we have a very definite clue to practically all the animal's behaviour.

a. Methods.—For rough, general work with mechanical stimuli a needle or a sharp-pointed scalpel may be used as the stimulating agent. For the finer work in sharply localising the stimulus, I at first made use of pieces of glass tubing drawn out to capillary fineness. This method was not, however, satisfactory, as the glass was too stiff to admit of reaching all points of the body under some circumstances. Furthermore, this stiffness, together with the sharpness of the end, made it almost impossible to give the animal a moderately strong stimulus without wounding it. A far better plan was found to be to fasten with sealing-wax a moderately stiff piece of human hair to a piece of glass tubing, the latter to serve as a handle. With such an arrangement the stiffness of the stimulating point can be varied by varying the length of the hair. Danger of wounding the animal is avoided, yet repeated strong stimuli may be given, while, further, the flexibility of the hair makes it possible to stimulate the animal at any point and from any desired direction.

An annoying difficulty in connection with this work was the clinging of the slimy secretion of the body to the point used for stimulating. Once coated with this slime the

sharpest point will slide off the body without giving any effective stimulation.

b. Description of Reactions.—The reactions can best be described by taking up in order the typical results following stimulation of the different parts of the body.

1. *Stimulation of Head Region.*—If a planarian gliding along on the bottom of a dish be touched with a needle on one side of the head, it will, under normal circumstances, in the majority of cases, turn the head and anterior one fourth of the body away from the side stimulated, and continue gliding along in the new path determined by the turning of the anterior end. This “turning away” reaction, or, as we may call it for economy of words, negative reaction, will always be given if the stimulus is made sufficiently strong. There is a certain intensity of stimulation below which the negative reaction may or may not be produced, depending on the physiological condition of the individual, but above which it always occurs. If, again, a normally gliding planarian be selected for stimulation, and this time the stimulating point (preferably something finer and more flexible than a needle) be touched very lightly to the edges of the sides of the head or the auricles, we get, provided the specimen is in the proper physiological condition, a very graceful and striking reaction, quite different from that obtained in the former case. This time the flat-worm will stop for the briefest instant, turn the head and a little of the anterior end of the body towards the side stimulated, and at the same time raise the head from the bottom, until finally the tip of the head points exactly towards the point from which the stimulus came, and then glide forward in that direction. This “turning towards” or positive reaction is given only in response to very weak mechanical stimuli, and then only when other conditions are favourable. It is a very precise and characteristic reaction when it does appear.

Having outlined the two main reactions following mechanical stimulation in the head region, we may proceed to consider each of them in more detail.

a. **Reactions to Strong Stimuli.**—The negative reaction is the characteristic reaction given to all strong stimuli, whether mechanical or of some other sort. It is, further, the same type of reaction which most organisms with fairly well-differentiated reactions give in response to strong stimulation. It takes the animal away from what might be a dangerous object.

In *Planaria* the portion of the body which takes part in the turning away varies with the strength of the stimulus to a certain degree. Stimuli just strong enough to call forth the negative reaction will cause only the head to be turned away. The first turn away of the definite reaction never in-

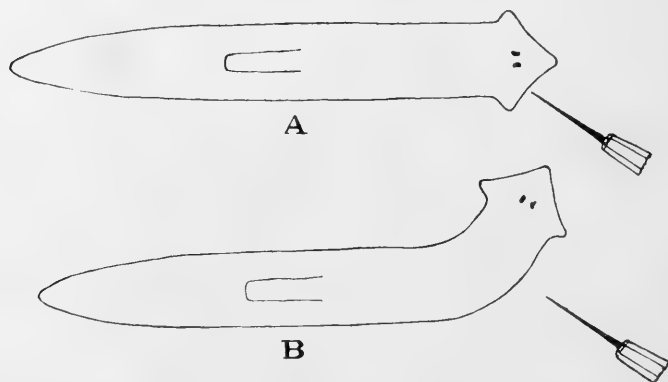


FIG. 14.—Diagram showing the form of the negative reaction to mechanical stimuli. A shows the position just before the stimulus is applied, and B the position after the reaction.

cludes any of the body back of the pharynx, so far as I have observed, except in the case of very strong and repeated stimuli. In the typical and most often observed form of the negative reaction the portion of the body which turns away is that anterior to a point about halfway between the level of the eyes and the point of origin of the pharynx. This is shown in Fig. 14. With stronger stimuli the point of turning is farther back on the body.

The number of degrees through which the head is turned in the negative reaction depends on the intensity of the

stimulus. With stimuli just effective in calling forth the reaction the turn is only slight, and since it affects only the head end the direction of movement of the whole animal may be scarcely changed at all. The amount of turning of the anterior end is typically from 30° to 40° .

There is in the negative reaction a pause at the instant of stimulation, preceding the turning away. The first effect of the stimulus is to cause the animal to stop its relatively rapid movement. This pause may be so slight as to be almost imperceptible in the case of comparatively weak stimuli, or, on the other hand, may lengthen to a quite noticeable interval when the stimulus is very strong. It is a characteristic feature of both the positive and negative reactions of planarians, and is evidently due merely to the fact that before a reaction (i. e. something involving a change of motion) the former movement must stop.

The effect of localisation of mechanical stimuli in the head region may next be considered. As has already been mentioned, stimulation of the sides of the head produces the positive or negative reaction according to the intensity of the stimulus. There are no special regions of specific sense-organs connected with either of these reactions. The negative response is given after strong stimulation of any part of one side or the other of the head and, so far as it is possible to observe, just as decidedly after stimulation of one part as of another. It is of interest to know what happens after stimulation of the head in the median line. It is very difficult to get a stimulus exactly in the median line, but one may come very near it by stimulating the dorsal surface of the head in the region between the eyes. The reaction produced is a longitudinal contraction of the anterior part of the body, drawing the head back away from the stimulus. The head is then turned to one side or the other as in the usual negative reaction, and the animal starts ahead again in the new direction. The side towards which the turn is made after median stimulation is indeterminate—that is, there is no tendency to turn in more cases towards one side than towards

the other, as has been found by Frandsen (:01) to be the case with *Limax*. This is what would be expected in the case of the flat-worm, because it is a perfectly bilaterally symmetrical organism. Probably what actually determines which way the organism shall turn after attempted median stimulation is the fact that the stimulus really acts a little to one side or the other, and the turning is really the negative reaction.

By repeatedly stimulating the anterior end of a worm with moderately strong mechanical stimuli its reactions may be modified. In the beginning of such a series of stimulation the worm turns away farther and farther from each succeeding stimulus, at the same time remaining at the same place in the dish, i. e. not making any progressive movements. This process tends to make the animal describe a circle away from the stimulus, about its posterior end as a fixed point. It never completely describes a circle, however, but after several stimuli have been given, to which it has responded progressively more vigorously, it finally jerks back with a strong longitudinal contraction, and turns the anterior end through a considerable arc, so that it points in an entirely different direction. This final strong reaction in the majority of cases turns the anterior end towards the side from which the stimulation is coming, or, in other words, in an exactly opposite direction to that of the previous reactions. This reaction appears as if, after the animal has tried in vain to get away from an uncomfortable stimulus by its ordinary reaction, it finally tries a wild jump in the opposite direction. This curious change in the reactions induced by a repetition of strong stimuli I have observed many times. It indicates the effect of the organism as a whole on its reflexes. As von Uexküll (:00, p. 73) has well brought out, we must consider that in the case of a higher organism, like a dog, the animal moves its legs, while with a lower organism whose activities are reflex—for example, the sea-urchin—it is really the “legs” (i. e. locomotor organs) which move the animal. In the flat-worm the movements of the

whole organism are determined by definite stereotyped reflexes, yet in such exceptional cases as the one just described the organism as a whole takes control, and does something quite different from what the normal reflex fitted to the case would accomplish.

Very strong mechanical stimulation of the anterior end, such as to wound the animal, causes a very much more vigorous reaction than the ordinary negative one, and of a slightly different form. The animal contracts strongly longitudinally, and, as a result of the heavier musculature on the ventral surface, curls the head in under the body. Then the anterior end is turned to one side through a larger angle than is usually the case, and the worm straightens out in this new direction. The point of importance to be noted in this reaction to maximal stimuli is the curling under of the head. The turn away from the side stimulated frequently is so great as to turn the animal squarely about, so that it heads in the direction opposite to that before stimulation. Besides this effect of maximal stimuli just described, they may also produce a change in the movement from gliding to crawling. The crawling does not usually follow stimulation of the head end of the body, but it is possible in some cases to produce it by very strong stimulation here. I have also been able in a normal animal to induce crawling backward by very strong and continued stimulation of the anterior end of the body. This backward crawling, when it occurs, is of the same character as the same movement in a forward direction, except that all the factors are reversed. It has been described above (cf. p. 551). It is much more easily produced after certain operative procedures, and in connection with them further details regarding it will be brought out.

The negative reaction, i. e. that to strong stimuli, is given more frequently than any other in the course of the activity of the individual, and apparently does not depend on the presence of any special physiological condition. It is given in response to stimuli covering a wide range of intensity. The lower liminal value of the stimulus producing it (there

is apparently no upper limit) varies to some extent with the physiological condition of the individual. Thus in some specimens at certain times stimuli which would ordinarily produce a rather strong negative reaction will call forth nothing but the positive reaction. This condition is only a transitory one, and the reason for it seems to be a heightened tonic condition of the animal. Specimens exhibiting this relation to rather strong stimuli are always very active, and move about with great rapidity, frequently raising the anterior end of the body and waving it about through the water as they glide along. Persistent strong stimulation of the organism rapidly changes the general physiological condition. This is not more true of stimulation applied to the head region than of strong mechanical stimulation of any part of the body. The animal becomes "stirred up" generally, moves about with increased rapidity, its sensitiveness to stimuli becomes diminished, and it will give only the negative response to stimulation of the anterior end. This change in the physiological condition of the animal as a result of continued stimulation of any sort, as in a series of experiments, is a matter of great practical importance in connection with reaction work. One may get totally different appearances from an individual which has been "stirred up" from what are seen in the case of one which is in the normal condition. This is only one of a number of factors which must be taken into account in work on the reactions and behaviour of an organism if one is to obtain trustworthy results. It is almost an absolute necessity that one should become familiar, or perhaps better intimate, with an organism, so that he knows it in something the same way that he knows a person, before he can hope to get at even an approximation of the truth regarding its behaviour.

β . Reactions to Weak Stimuli.—The positive reaction is the characteristic reaction given to all weak stimuli. It is an orienting reaction in the sense that it brings the anterior end of the animal in a position such that it points approximately towards the source of the stimulus. On

account of its fineness of adjustment with reference to the strength of the stimulus and the general physiological condition of the animal, it is a response which might be very easily overlooked in a superficial examination of the behaviour. As the worm gives this positive reaction in response to a gentle stimulus, turning the head towards the source of stimulation, and at the same time raising it, it gives one the impression that it is seeking something, and such the behaviour would doubtless be called by some animal psychologists. This impression is enhanced by the fact that if the head does not come in contact with the stimulating object at the first reaction, the animal advances in the direction from which the stimulus came, with the anterior part of the body raised and waving from side to side in the water.

As has been mentioned, the reaction is very delicately adjusted physiologically. In the majority of cases the animal must be in a comparatively quiet condition,—that is, not “stirred up” or excited, and gliding smoothly at the ordinary rate, in order that the reaction may appear at all. The stimulus must ordinarily be very weak, and given so as not to disturb the animal by abruptly changing the surrounding conditions. It is possible to produce the reaction by the use of a needle or scalpel point if sufficient care is taken, but better results are obtained by the use of a hair as the stimulating point. The point should be lightly touched to the edge or dorsal surface of the head, and then quickly drawn a short distance away. Even when all these precautions are taken one may fail to produce the characteristic response. I have frequently found that the same specimen which at one time would give the positive reaction in a very definite and characteristic way to every light touch on the head could not be made to show it a few hours later. This shows how closely it depends on general physiological conditions. On the other hand, specimens will frequently be found that for short periods of time (two or three hours) can hardly be induced to give any other response to mechan-

ical stimulation of the head. Stimuli strong enough to be far above the usual upper liminal value for this reaction will call it forth. Such specimens show the reaction in a much more pronounced type than is usually the case. After a stimulus has been given they will turn towards it, and if the source is not touched immediately they will remain in the same spot waving the head about the region from which the stimulus came, at the same time stretching the anterior end of the body far out in all directions, precisely as if in search of the stimulating body. Usually this hypersensitive condition passes off in a short time, and the animals behave again in a more normal fashion. It was thought that possibly this condition was due to hunger, but experiments¹ devised to test this question indicated that this was not the case. We can only say that it is due to some intimate physiological condition, the exact nature of which we do not know. Another fact which may be mentioned in this connection is that sometimes a specimen in normal condition will give the positive reaction in response to a certain strength of stimulus only a part of the time. Other trials result in entire indifference on the part of the organism. Of course, it is not possible to give mechanical stimuli always of the same strength, yet with the closest possible approximation to this by an experienced operator, some of the trials will not affect the animal in any way except to cause a slight local contraction at the point on the head stimulated. The worm glides along without any change in rate or direction. Altogether we must conclude that the reaction is one which is very closely dependent on the existence of certain definite internal conditions as well as the external ones.

The typical course of the reaction is, as has been described, first a momentary pause, followed by a turning of the head towards the stimulus, accompanied by a raising of the anterior part of the body. From this typical form of the reaction there are many variations. The raising of the anterior end from the bottom just before and during the time it is being

¹ See section on "Reactions to Food and Chemicals."

turned towards the source of the stimulus may be entirely omitted. In this case the head is swept around towards the stimulus without being any further raised from the bottom than in the ordinary glide. The duration of the pause immediately following stimulation is likewise subject to great variation. It may be so diminished as to be imperceptible, the worm sweeping the anterior end around through the water without any change in the rate of the glide. The amount of the turn varies with the point of application of the stimulus, the head being turned just far enough to point in the direction from which the stimulus comes. This orientation, if we may so call it, is generally quite exact. If the stimulus is near the middle line on the edge of the head the turn will be only through a few degrees, while if the auricles are touched it will amount to nearly 90° . This fact indicates the remarkably well-developed co-ordination of the reaction. There is a great deal of variation with regard to what takes place after the turn has been made, and the anterior end is directed towards the stimulus. If the stimulating point is removed immediately after stimulation, so that the animal does not touch it by means of the first reaction, a normal specimen will usually lower the head and continue gliding in the new direction. As has been mentioned, however, in some cases a specimen will continue "feeling" about in the locality for some time. If the stimulating point is held in about its original position after the stimulus has been given, the first reaction will in most cases bring the head into contact with it. In this event the animal usually moves the tip of the head about over the hair (or other point) for a short time, and then drops back to the bottom and continues gliding. In other cases it will clasp the anterior end about the hair (as in the feeding reaction to be described later), and then in a moment start gliding up over it. When this happens the hair or needle may be moved about in the water or even lifted out of it, and the animal will not let go its hold and drop off. If the needle is held quiet, however, the animal will in a short time glide down off it and proceed on

its way along the bottom. This behaviour when the animal is able to reach the stimulating object is evidently the action which most frequently occurs in natural environmental conditions.

With regard to the localisation of the stimulus producing this reaction, I may say that I have been able to produce it by proper stimulation of any part of the edge or dorsal surface of the head region under favourable circumstances. It seems to be more certainly produced—that is, in a larger number of cases—by stimulation of the auricles than of any other part of the head, and it may be that in this is to be found an indication of the chief function of these sense-organ bearing structures. At any rate, this is the only indication of a special function for them which I have been able to discover. The positive reaction given in response to light stimulation of the dorsal surface of the head is necessarily somewhat different from the typical reaction which has been described. In this case there can be no turning towards one side, because if this were done the head would not be directed towards the source of the stimulus. Instead, what takes place is this: the head is sharply raised and twisted, so as to form a part of a spiral in the region posterior to the head. This brings the anterior end into a position pointing towards the source of the stimulus, and at the same time the ventral surface is brought around so as to be, in most instances, the first portion of the body to touch the stimulating point. This reaction, following stimulation of the dorsal surface of the head, is not an easy one to obtain. I have succeeded best in producing it in the case of individuals in the hypersensitive condition mentioned above.

With regard to the strength of the stimulus necessary to call forth the positive reaction, only very relative statements may be made. Unfortunately we have no method of measuring the intensity of such weak mechanical stimuli as are used in work on lower organisms. Our only idea of the strength of the stimulus must come from the reaction of the organism itself. It must suffice to say, regarding the reaction

under discussion, that in an animal in a condition of hypersensitivity I have been able to produce the reaction by the weakest stimuli which I was practically able to give. Under normal conditions of sensitiveness it takes a slightly stronger stimulus. No absolute value can be given for the upper limen of the reaction, beyond which it does not appear, but gives place to the negative reaction. This value varies greatly with different individuals. The general statement may be made that the positive reaction is the characteristic response to stimuli of very low intensity, and its production is very closely dependent on the proper gradation in the intensity. This dependence is so close that it is possible to obtain a part of both the negative and positive responses combined in the same reaction by the use of a stimulus of the proper intensity. I have been able in a few very favourable cases to produce by a single stimulus a pronounced raising of the head, such as is characteristic of the positive reaction, followed by a turning away from the source of the stimulus. Now the raising of the head is no part of the typical negative reaction, and, furthermore, was done in the very characteristic way in which it occurs in the positive reaction. The stimulus which produced it was evidently about intermediate in intensity between what, in the case of the particular animal used, would have called forth either the positive or negative reaction, as the case might be. This experiment shows in a very striking and conclusive way that in both the positive and negative reactions we are dealing with a complex of reflexes, since here a part of one of the reactions is associated with a part of the other. This point will be alluded to again in another connection, and its significance more fully pointed out.

The evident purposeful character of the positive reaction is plainly apparent. It is a reaction admirably suited, on the whole, to bring the organism into contact with beneficial things, such as food, etc. It seems to me that it must be by far the most important reaction of the animal in the struggle for existence. In the conditions under which planarians

live a reaction which gets it food, or helps to, is of far greater importance for the survival of the individual than a reaction which takes it out of danger; for, so far as observation can show, the dangers it encounters are relatively few. It does not move over large areas of territory, and, so far as is known, it does not furnish a considerable part of the food supply of any other organism. Altogether its chief struggle for existence would seem to consist in obtaining subsistence for itself, and for this the positive reaction to mechanical stimuli would appear to be an important aid. As will be shown later, the food reaction proper consists largely of this same response.

We may now pass to a consideration of the—

2. Reactions to Stimuli applied to the Middle Region of the Body.—I use the term “middle region of the body” to distinguish that portion extending from the posterior border of the head to about the middle of the pharynx. The separation of the body behind the head into a “middle” and a “posterior” region is based entirely on physiological considerations, and is not defined morphologically.

a. Reactions to Strong Stimuli.—Strong mechanical stimulation of the middle region of the body along the lateral edges causes, in the first instance, a local contraction of the body in the immediate region of the stimulus. This local contraction is well marked; much more distinct than that in the head region. If the stimulus is sufficiently strong, and especially if the stimulating point is applied to the edge from above rather than from the side, the previous gliding movement will be changed to crawling. This will continue for a brief interval, usually from two to four crawling contractions being given; then the animal will relapse again into the glide, provided the stimulus is not repeated. In the case of a strong stimulus applied to the side of the middle region of the body, especially if the stimulus is several times repeated, we get the negative reaction—a turning away from the side stimulated—just as in the similar

case in the head region. The nearer to the anterior end of the middle region the stimulus is applied, the more easily will the negative reaction be produced, while back in the pharyngeal region it follows even strong stimulation in fewer cases. In all cases where this reaction is not produced the direction of movement is either unchanged by mechanical stimulation, or the anterior end may be brought around very slightly towards the side stimulated as a mechanical result of the local contraction on that side. By repeating the strong stimuli on one side of the middle region, summation effects similar to those described above as taking place when the head is similarly treated are not produced. The animal crawls faster and faster away from the stimulus. Its direction of movement is changed, but usually not more than thirty to forty degrees. We see here evidence of precise response to localisation of stimulus. Stimulation of the head causes the animal to turn to one side, and, in case the stimulus is very strong, to contract longitudinally strongly before doing so. As we go back from the head in the middle region of the body, the tendency to crawl rapidly ahead away from the stimulus increases. At the same time the turning away from the stimulus becomes less and less marked the farther back it is applied. In no case do we get any strong retraction of the anterior end, which, in case of stimulation of the middle region of the body, would tend to bring the animal, or at any rate a part of it, into further contact with the stimulus.

Strong stimulation applied to the dorsal surface of the middle region of the body causes the animal to change from the glide to the crawl. This change of the form of motion may be regarded as a specific reaction in response to strong stimulation of the middle or posterior regions of the body. Stimulation of the dorsal surface of the middle region does not change the direction of the movement unless the stimulus is applied near the lateral margin, in which case it may cause the negative reaction, as mentioned above.

β. Reactions to Weak Stimuli.—Weak mechanical

stimulation of the sides of the middle region of the body causes, in the first instance, a small local contraction at the place stimulated, without any effect on the general direction of movement of the whole organism. Under favourable circumstances, however, it is frequently possible to get a quite different result by the use of a weak stimulus on the lateral margins of this region. Specimens of *Planaria* may be induced to give the characteristic positive reaction described above by stimulation of a point as far back as the middle of the pharyngeal region. The stimulus inducing it is of the same intensity and character as that which will produce the same result in the head region.

Some experiments bearing on this point will be reported in full, on account of their important bearing on theoretical questions to be taken up later. These experiments were to

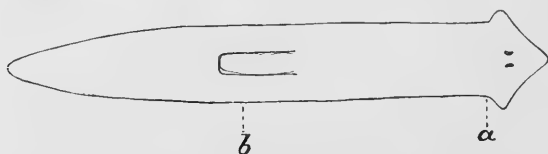


FIG. 15.—Diagram to show the portion of the side of the body (*a b*) within which weak stimulation produces the positive reaction.

test the effects of weak stimuli on the sides of the middle region of the body, especially with reference to the relation of the physiological condition of the individual to its reactions. The experiments were performed on large specimens of *P. maculata*, and the region of the body stimulated was that from *a* to *b* in Fig. 15. Most of the stimuli were applied in the region between the auricles and the origin of the pharynx. The stimuli were given by moving the point of a fine scalpel along the bottom of the dish till it came into contact with the margin of the body. In this way no general disturbance was produced. The attempt was made to make the stimuli of as nearly as possible the same intensity each time. The results were classed as positive, negative, or indifferent, according as the

specimens gave the usual positive or negative reaction, or kept their course without change, showing only the local contraction. The first series which I will report was on an individual which was in a condition of great excitation, moving about with more than normal rapidity, and generally "stirred up." The results of twenty-three stimulations on this specimen were—

Expt. I. Positive responses . . .	1	} Specimen in state of excitation.
Negative responses . . .	0	
Indifferent responses . . .	22	

A similar experiment with another individual in an entirely normal unexcited state, gliding at a moderate rate, gave the following results :

Expt. II. Positive responses . . .	20	} Specimen in normal condition.
Negative responses . . .	2	
Indifferent responses . . .	13	

The striking preponderance of the positive reaction in the case of the unexcited individual is notable. The same individual used in Experiment II was now "stirred up" by poking it violently about the dish with a needle for about five minutes. It was then allowed to settle into a glide which was at a more rapid rate than normal, and another series of stimulations was made, with the following results :

Expt. III. Positive responses . . .	1	} Specimen in condi- tion of excitation.
Negative responses . . .	3	
Indifferent responses . . .	8	

Here, again, the indifferent responses are in excess, and there are practically no positive reactions. The specimen was again "stirred up" in the same way as before, and another series taken.

Expt. IV. Indifferent responses (trials 1 to 11 inclusive)	} Specimen in state of excitation.
Positive responses (trials 12 and 13)	
Negative responses (0) . . .	

The specimen was again stirred up in the same way and another series taken, with the following results:

Expt. V. Indifferent responses (trials	} Specimen in state of excitation.
1 to 9 inclusive)	
Positive responses (trials 10 and 11)	
Negative responses (0)	

The positive responses in all these experiments were very definite and characteristic. I have obtained the same results in many other series of experiments, which need not be recorded in detail. The experiments show very clearly that in order for the animal to give positive responses to weak stimuli it is necessary that it be in an unexcited condition. These results have also an important bearing on the question of the mechanism of the positive response, in that they show conclusively that the reaction does not depend on the stimulation of special sense organs located in the head regions alone.

Weak mechanical stimulation of the dorsal surface in the middle region of the body is usually without any effect other than the causing of a slight local contraction at the point stimulated. If any specific effect on the whole animal is produced, it is merely a change from the gliding to the crawling movement, such as results from strong stimulation in the same region.

3. Reactions to Stimuli applied to the Posterior Region of the Body.—By “posterior region of the body” I mean that part of the body from the pharyngeal region to the posterior end. This region is not sharply marked off physiologically from the middle region, and it is impossible to say in any given individual at just what level the demarcation will be found. The physiological distinction between the two regions is founded on the fact that it is possible by unilateral stimulation of the middle region of the body to produce a change in the direction of the movement of the animal as a whole, while in case of the posterior region,

as will be shown, this cannot be done. On this account it will not be necessary in the description of the reactions to sharply distinguish between the effects of stimulation of the margins and of the dorsal surface, as has been done in the previous cases.

Strong mechanical stimulation of the posterior region of the flat-worm produces as a specific reaction an immediate change from the gliding to the crawling movement. The direction of the crawling is the same as that of the gliding; that is to say, the worm keeps on in a straight line, taking itself directly and in the quickest possible way away from the stimulus. The duration of the crawling movement following stimulation of the posterior region varies with the relative intensity of the stimulus and the physiological condition of the specimen. The most usual number of the strong, crawling contraction waves following strong stimulation is three or four. We may get a smaller number than this, and very frequently do, but in the species studied I have very rarely seen more than four of the general contractions following a single stimulus. This is evidently all that would be necessary under normal circumstances, since four of these strong contractions will carry the animal a considerable distance ahead, and probably out of reach of the stimulating agent. The weaker the stimulus is, the fewer are the contractions and the shorter the distance crawled. In some individuals it is at times almost impossible to induce the crawling movement except by repeated stimulation. Such specimens will merely draw up the posterior end in a single crawling contraction after stimulation, and then immediately relapse into the glide. If a strong stimulus is repeatedly given at the posterior end the crawling is continued, becoming more and more rapid. This is the only effect of continued stimulation in this region, there being no summation effect corresponding to that produced by stimulating the anterior end. No different effect is produced by stimulating the margins of the posterior region of the body from what takes place when the point stimulated lies near

the middle line. There is no turning towards or away of any part of the body. The lack of any special effect of unilateral stimulation is not surprising, for the reason that rapid movement in a forward direction will get the animal away from harmful stimuli affecting this region, in the long run, more quickly than any other. Further, there would be no advantage in the production of a positive reaction by stimuli at the posterior end. If we think of these reactions as having been developed by natural selection there would be no possibility of such a reaction having arisen, for the reason that practically any favourable stimulus would be encountered by the anterior end before it possibly could be by the posterior. Very weak mechanical stimulation of the posterior end of the body causes only a local contraction at the point stimulated.

4. Reactions to Stimulation of the Ventral Surface.—In the descriptions of the reactions to mechanical stimuli up to this point we have been considering stimuli applied to the dorsal surface and to the margins of the body. It may be well to describe briefly what the reactions in response to localised stimulation of the ventral surface are. This matter can best be tested when the animal is moving on the under side of the surface film, with its ventral side uppermost. It might be supposed before the trial was made that this habit of the animal would afford ideal conditions for testing its reactions to ventral stimulation, but, as a matter of fact, the conditions are anything but ideal. The flexibility and elasticity of the surface film makes it almost impossible to touch it with a stimulating point anywhere within a radius of a centimetre about a planarian without causing the animal to be jerked bodily to one side or the other, quite sharply and for some little distance. This is, of course, a mere mechanical effect, which takes place with lifeless bodies also. Furthermore, as has been mentioned in an earlier section, it appears to be very difficult for planarians to quickly change the direction of their movement when on the surface film (as is necessary in reacting to stimuli). On account of these

conditions it is very difficult to get any certain and trustworthy results from the stimulation of the ventral surface. My results have been as follows:—strong stimulation of the anterior end on one side of the middle line causes the negative reaction just as when the stimulus is applied at a corresponding point on the dorsal surface. For mechanical reasons the response is not as extensive as when the animal is on a solid, but there seems no doubt of its character. The positive reaction to weak stimuli I have not been able to produce in any certainly recognisable form in response to stimulation of the ventral surface, but I think this negative result is due probably to the external conditions, and not to a real failure of the organism to react. Strong stimulation of the posterior end of the body causes the gliding to change to the crawling just as under other conditions. Very strong mechanical stimulation of the ventral surface of the body causes the animal to let go its hold and pass down to the bottom.

5. Reactions of Resting Specimens to Mechanical Stimuli.—A resting specimen gives no response whatever to weak stimuli which are still strong enough to produce a definite reaction when the worm is in the active condition. The stimulus is simply below the threshold of the resting animal's sensitiveness. To stronger stimuli the reactions correspond in form with those given by the active animal, but are less pronounced. For example, rather strong stimulation at the anterior end induces a weak negative reaction; similar stimulation of the posterior end sets the animal off into the crawling motion. Strong stimulation of any part of the body besides producing the characteristic reaction for that region (that is the negative reaction) will also in most cases start the animal into movement. This will always be the case if the stimulus is of sufficient strength, or is several times repeated. As would be expected from the low sensitiveness of the resting flat-worm, it is impossible to call forth from it any positive reaction.

6. Reactions to Stimuli given by Operative Pro-

cedure.—Evidently when a planarian is cut the cutting induces a strong stimulation, which is of the same kind as that induced by ordinary mechanical stimuli, only much more intense. The immediate effects of operations may then be taken up in this section.

If we take first the typical case given by cutting the animal transversely in two in the region between the posterior border of the head and the origin of the pharynx, and make the cut by a single stroke of a sharp scalpel, we find that the effect on the anterior piece is precisely the same as that of an ordinary strong mechanical stimulation of the same place. That is, this piece merely changes from the gliding to the crawling movement, and after giving three or four crawling contractions settles down again into the glide. This is the same result essentially as that obtained by Norman (:00) and earlier by Loeb ('94 and :00). In the behaviour of the posterior piece in this experiment under discussion there is a great deal of variation. In about 70 per cent. of all cases in which I have observed the results of such an operation, the posterior piece crawled backwards as a result of the cut. In the remainder of the cases the piece either stayed in the same place and contracted violently, or else glided ahead. The amount of the backward crawling when this occurs varies greatly, from a short distance involving only one longitudinal crawling contraction to several times the length of the worm, the movement lasting in this latter case for over a minute. In order that this backward crawling may appear in a well-marked and distinct form it is necessary that the posterior piece be above a certain size. Very small posterior pieces after operation usually remain quiet.

A cut so made as to split the anterior end of the body in the middle line in most cases causes the worm to crawl backwards just as does a transverse cut. In some cases this, as well as other operations, merely causes the animal to contract violently and squirm about at the same place. Splitting the posterior end of the body in the middle line causes the parts

on either side of the cut to give violent longitudinal contractions, while the worm as a whole starts crawling ahead; that is, it changes from the gliding to the crawling movement.

Oblique cuts produce essentially the same effects as would transverse cuts in the same part of the body, i. e. forward crawling of the anterior piece, and usually backward crawling of the posterior piece. This is true unless the cuts are very oblique, so as to form very acute angles with the sagittal plane of the body. In such cases the effects produced more nearly resemble those obtained in complete longitudinal splitting of the body. If the body is split completely into two parts longitudinally, there is usually very little progressive movement of either piece afterwards. The pieces contract strongly on the cut sides very soon after the operation is performed, so that they take on the form of a bow, which in many instances becomes a nearly complete circle. This being the case, any progressive movement, either by gliding or crawling, is nearly or quite impossible. Cuts involving only a small portion of one side of the body produce, if in the anterior region, the characteristic negative reaction given to other strong mechanical stimuli, while if in the posterior region they cause the crawling ahead.

Cuts made on the resting animal produce essentially the same effects as on the gliding specimen. Unilateral cuts have the same effect in producing the negative reaction.

7. The Effect of Mechanical Hindrance to Movement.—A series of experiments was performed on *Dendrocœlum*, sp., with reference to the behaviour of the animal when progressive movement was made impossible, and yet the animal was stimulated strongly at the same time. These conditions can be realised by thrusting a needle through the centre of the body from above, and then holding it fixed in position. The results of this procedure varied somewhat, according to the portion of the body through which the needle was thrust. In case the hindrance is in the posterior region of the body, e. g. at a point just behind the posterior

end of the pharynx, the effect immediately following the thrusting in of the needle is a strong longitudinal contraction of the whole body. After this first strong contraction the animal remains perfectly quiet in the contracted form for a varying length of time (in some cases as long as five minutes, but usually less). After this period of quiet a series of rhythmical waves of contraction pass longitudinally over the still contracted body. The purpose of these waves is evidently to loosen the restraining object by making the hole in the body through which it passes larger. This is the same behaviour that I have observed in the deposition of the large egg. This process of rhythmical longitudinal contraction is continued for a time; then the animal stretches to its extreme length, attaches the anterior end to the substrate, and attempts to crawl away. The movement of the anterior end is precisely the same as in crawling. The animal turns and twists and struggles violently in this attempt to crawl away, and the cilia beat strongly. If the needle occupies a position near the edge of the body this first struggle will usually be sufficient to tear the body loose from the needle, so that the animal may then move ahead freely. Such specimens will, of course, have a large jagged wound in one side of the body, which, however, closes in and heals in a short time. In case the first struggle of the extended animal to crawl ahead is not effective, that is if the needle is too far in towards the centre of the body to make the tearing out possible, the animal, after continuing the struggle for a time, contracts strongly longitudinally and goes through the whole series of stages of quiet, rhythmical, longitudinal contraction and attempted crawling again. The only difference between the first and succeeding series of trials is that the stages in which the animal is strongly contracted longitudinally tend to become shorter with each repetition.

In case the needle is thrust through the body in front of the pharynx, the strong longitudinal contraction appears as before, and is followed after some time by an extension of the part in front of the needle, while the rest of the body re-

mains quiet and contracted. This short anterior region, including hardly more than the head, goes through the crawling movements, but on account of its small size is very ineffective so far as pulling the body away from the needle is concerned. In my experiments I have never seen any worm succeed in getting free from a needle put through the body in this position.

This general behaviour of the animal in response to restraint of movement is very interesting, especially in the cases where the restraint is at the posterior end, as showing the relation between the behaviour and the capability of regenerating. The organism tears itself loose from a restraining body with entire nonchalance, as it were, and its confidence is well founded because no permanent harm comes from the action. The lost and wounded parts are regenerated and healed in a short time. The behaviour takes advantage of the ability to regenerate. Whether the form of behaviour (pulling away from restraining objects) or the power of regeneration and reparation appear in the organism first we cannot say, for either might very well follow, in a more or less remote causal connection, the other. What we do know is that at present there is a very nice condition of mutual adaptation between the two things.

The effect of the hindrance of a rather light weight at the posterior end of a worm is to induce the crawling movement. This can be seen in case the animal is feeding on a small piece of food material, and, as frequently happens, starts into movement before the pharynx is withdrawn. The piece of food attached to the end of the pharynx is dragged along behind, and the movement is the crawling. Frequently, also, in feeding experiments pieces of food will get stuck to the posterior end of the worm by means of the mucous secretion of the body, and these have the same effect in inducing the crawling movement.

Having now obtained a descriptive basis we may pass to a discussion of some general features of these reactions. We may first take up—

c. *The General Features of the Reactions to Mechanical Stimuli.*—From the above description it appears that the nature of the reactions to mechanical stimuli depends upon several factors. These are—

1. The intensity of the stimulus.
2. The localisation of the stimulus.
3. The physiological condition of the organism.

The reactions given may be of several different kinds, depending on the factors mentioned above. These are chiefly as follows:

1. The resting individual may begin locomotion.
2. The gliding movement may be changed to the crawling movement.
3. The forward movement may be transformed to movement backward.
4. The animal may turn away from the source of the stimulus (the “negative” reaction).
5. The animal may turn towards the source of the stimulus (the “positive” reaction).

It is evident that the reactions last named—the negative and positive reactions—are the most important and most interesting from the theoretical standpoint. It is of the greatest interest to note that these two qualitatively opposite reactions are induced merely by differing intensities of stimuli, the stimuli being otherwise identical throughout.

It is to be noted further that the positive and negative reactions have the characteristics of purely reflex acts. Each reaction has a perfectly definite and characteristic form. While, in some cases, which of the two reactions will be given in response to a particular stimulus depends on the physiological condition of the organism, yet it is practically always either one or the other of the typical reactions. Only very rarely do we get any deviation from the type forms, and in such cases the reaction is evidently a combination of easily recognisable components of the two typical complexes of reflexes.

These two reactions are evidently not single simple

reflexes, but are complexes of several simple reflex acts. It may be well to present in tabular form the different components in each of these reactions, indicating by the position in the table the relations of the parts.

Component Phases of the Reactions to Mechanical Stimuli, with special reference to the Head Region.

POSITIVE.	NEGATIVE.
A. Momentary stopping of previous movement. Referred to as "pause" or "hesitation" in description.	A. Same as in positive.
B. Longitudinal extension of the anterior end to greater or less extent. Amount depends on previous extension. Usually distinctly noticeable.	B. Longitudinal contraction of anterior end of greater or less intensity. Tends to make A appear more pronounced and longer in duration.
C. Turning towards one side, viz. that stimulated. This side is defined by the position of the source of the stimulus, not structurally. Sharp "orientation."	C. Turning towards one side, viz. that not stimulated. Defined as in positive. No sharp "orientation."
C'. Raising of anterior end. This takes place at the same time as C.	
D. Movement towards stimulus. Direction determined by position taken by anterior end at termination of C.	D. Movement away from stimulus. Direction determined as in positive.

Time relations are indicated by vertical position in the table. Components occurring at the same time are included in braces.

Each of the components before D may be considered as a single reflex, and thus there are in one case four and in the other case three simple reflexes which go to make up the whole reaction. That these reactions are composites of the distinct parts is evidenced, first, by direct observation of the reactions themselves; and second, by the fact that it is

possible by varying the strength of the stimulus to produce only certain parts of the whole reaction without the remainder, and, furthermore, that a part of one reaction may in rare instance be combined with a part of the other (v. sup., p. 587).

d. Mechanism of the Reactions.—A question which is of the greatest importance in all work on the reactions of organisms is, what is the mechanism of the reaction? In the case of the flat-worm this becomes, what is the neuromuscular mechanism of the reactions? Very little direct evidence bearing on this question can be obtained from the reactions themselves. Taking the positive and negative reactions as they occur, there are several different sets of muscles and of nerve connections by means of which they might conceivably be brought about. The best evidence on the question is the indirect evidence from operation experiments, in which parts of the mechanism are injured or removed.

1. *Relation of the Brain to the Reactions.*—The first specific problem which may be taken up may be stated thus: is the brain necessary for the performance of the normal reactions to mechanical stimuli? Or, in other words, will a planarian from which the brain has been removed react normally to stimuli? This question can be answered from the study of specimens which have been cut in two transversely, and consequently we may proceed at once to a description of the reactions of the pieces resulting from such an operation. A typical specimen is cut in two transversely at the level of a point about halfway between the head and the origin of the pharynx, as shown in Fig. 16. As has been mentioned above, the cut itself acts as a strong mechanical stimulus, and the immediate effect of the operation is to set both pieces crawling, the anterior one ahead and the posterior one usually backward.

If now the pieces are allowed some hours to recover from the immediate effect of the operation, and then stimulation is tried, the following results are obtained:—With the anterior

piece A, containing the brain, the results are entirely similar to those obtained in case of the normal animal. Strong unilateral stimulation of the head causes the negative reaction, weak stimulation of the same sort the positive reaction. Stimulation at the posterior end causes the crawling movement to appear, and altogether the appearances are essentially the same as in the normal complete specimen.

The posterior piece B (lacking the brain) behaves in a somewhat different manner. If the anterior end of this piece is given a stimulus of moderate intensity anywhere on the cut surface the piece will usually start crawling straight backwards. This is almost always true for a short time after the operation, and is especially well shown in such specimens as started crawling backwards as a result of the cut. When from twenty-four to forty-eight hours have elapsed after the operation this tendency of posterior pieces

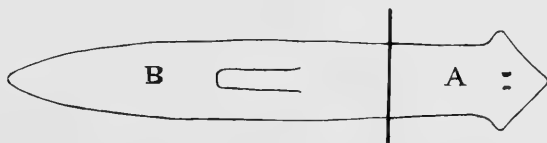


FIG. 16.—Operation diagram. Heavy line indicates cut.

to crawl backward on stimulation of the anterior end begins to grow less marked, and, as regeneration proceeds, finally disappears. In many such posterior pieces I have been able to produce this backward crawling in a very pronounced form, and of comparatively long duration (three or four minutes at a time). The character of the movement has been described above. If the stimulus is applied to one side or the other of the anterior end of such a posterior piece, instead of squarely against the cut surface, a well-marked negative reaction is produced; that is, the anterior end turns away from the stimulus just as a whole animal would. The reaction is very definite, and of precisely the same character as the normal negative reaction. The only difference to be observed is that in proportion to the strength of the stimulus the reaction is not so pronounced as in the

normal animal, this being due to the generally lowered tonus in such a piece. I have not been able to obtain any positive reaction (i. e. turning towards the stimulus) in such a posterior piece after operation. Stimuli which are at all effective produce the negative response. This experiment has been tried many times, but always with the same result ; the positive reaction never appears. If the posterior end of such a posterior cut piece is stimulated the crawling movement is produced just as in case of the normal complete animal. As has been noted in connection with the movement, there is a general reduction of tonus in the posterior pieces resulting from transverse cuts. This low tonus involves not only the motor functions, resulting in slower movement, but also to a less extent the sensory functions. Such a piece is somewhat less sensitive to mechanical stimuli than normally. The cut surface is more sensitive to mechanical stimuli than any other part.

Now it will be seen from the above description of the reactions of a piece from which the brain has been removed, that the most striking difference in the behaviour of such a piece from that of a normal animal is to be found in the absence of the positive reaction.

There are three conceivable possibilities as to the cause of the absence of the positive reaction in pieces from which the head has been removed. First, the positive reaction might be due to the stimulation of certain sense organs which are removed by the operation. But this is decisively negatived by the fact that in an entire worm stimulation of points posterior to the level of the cut removing the anterior end will cause the positive reaction.

Second, it might be conceived that the reaction is brought about by a special localised muscular mechanism, which is removed or destroyed by the cut. But there is no evidence of the existence of such a mechanism ; and further, it will be shown later that the ordinary musculature of the body, which is of course uninjured in the posterior part, is sufficient to bring about the reaction.

Finally, the positive reaction might in some way be a specific function of the brain, which is removed by the operation. As the evidence seems to be decisive against the first two possibilities this seems probably true. Is this because the brain contains a special "centre" whose function it is to produce the reaction?

There is no reason to think of the reaction as a function of the brain in the sense that that organ forms a centre which originates the impulses which cause the reaction. On the contrary, it seems much more probable that the loss of the brain causes the loss of reaction for the following reason. It has been shown that removal of the brain causes a general lowering of the tonus of the organism, and further that the appearance of the reaction in a normal animal is closely dependent on the tonic condition of the organism. Probably, then, the chief reason for the non-appearance of the positive reaction in posterior pieces is that in these the conditions of general tonus are so changed by the loss of the brain that the reaction is no longer possible. Expressing it in another way, the animal is too sluggish to give the positive response. This being the case, it would be expected that it might be possible to induce the positive reaction in a decapitated specimen provided the tonus were raised in some way. As a matter of fact, as will be shown later, positive reactions to certain chemical stimuli have been observed in a few cases (cf. p. 649). In its form and mechanism the positive reaction is not directly dependent upon the brain.

Summing up the evidence on the relation of the brain to the reactions of the flat-worm, it may be said that all the reactions to mechanical stimuli shown by the normal animal, with the single exception of the positive reaction, are given by specimens from which the brain has been removed. The relation of the brain to the positive reaction is, in large part, so far as evidence can be obtained, an indirect one, viz. it is necessary for the maintenance of the proper tonic conditions of the organism. Thus far there is no evidence of any special "centre" functions of the brain, similar to those

supposed to exist in the cortical centres, for example, of a mammal.

2. The Neuro-muscular Mechanism.—In the negative reaction to mechanical stimuli the anterior end of the body is turned sharply away from the source of the stimulation, while in the positive reaction it is equally sharply turned towards the source. These relations immediately suggest the following questions:—Is the negative reaction the result of a crossed impulse, which, originating at the point stimulated, crosses over to the other side of the body and causes the contraction of the longitudinal muscles on that side, thus producing the turning away from the stimulus? What is the course of the nerve impulse which produces the positive reaction? What sets of muscles are concerned in the production of each reaction?

The discussion of the negative reaction may be taken up first. If the nervous impulse producing this reaction crosses the body to produce a contraction on the side opposite from the stimulus, the experiment cited in the section above shows that this crossing cannot occur entirely in the brain, but must also occur in some part of the body posterior to the brain; or at any rate, be capable of so doing in a quite normal fashion immediately after removal of the brain. In this experiment where the body has been cut in two behind the brain, the posterior piece performs the negative reaction in a quite normal way immediately after the operation. This experiment may be carried farther, and the animal cut in two transversely in places nearer and nearer to the posterior end of the body. In all of these cases, until the piece becomes too small to show definite movements of any sort, the negative reaction may be obtained by strong unilateral stimulation. This shows conclusively, then, that if the negative reaction is to be considered a crossed reflex, there must be all along the body a series of cross-commissures which are at all times ready to bring about in co-ordinated perfection a result with which they have never previously had anything to do. This conclusion seems in-

evitable because, as has been shown above, unilateral stimulation of the posterior region of the body in a normal individual does not cause the negative reaction, but instead merely causes the animal to move ahead faster by crawling. If these paths for the crossing of impulses which are so immediately effective after the operation are present in the uninjured specimen, one would expect the reaction to be of quite a different character from what actually occurs. A stimulus applied near the posterior end would naturally cross over at once and produce a bending on the opposite side at the same level. Or the stimulus might diffuse, so that the entire opposite side would be affected and the worm would become uniformly curved on that side. But as a matter of fact we find that the turning affects only the anterior portion of the body. If it is urged that after operation the crossing of impulses takes place through the general protoplasm the difficulties encountered are no less, for it must be shown how passage of an impulse through the protoplasm to cause a perfectly well co-ordinated reaction can appear so quickly and produce such perfect results at once. If tested immediately after the operation, before the general lowering of tonus is felt, the reaction time for the negative response of a posterior piece of the body will not differ appreciably from that of a normal worm. Now, according to the views of the advocates of the theory that after operations involving loss of nervous tissue, impulses may be conducted through the general protoplasm, it is held that such conduction is always at first appreciably slower than in nervous tissue. It would also seem on purely *a priori* grounds that this must be true.

Thus it is seen that there are serious objections to the view that the negative reaction is the result of a contraction on the side of the body opposite to that stimulated—that is, that it is a crossed reflex. The question now arises, if the reaction is not produced in this way, in what other way can it be produced? Evidently it is quite possible that the anterior part of the body can be turned away from the stimulus by a lengthening of the side stimulated, quite as well as by a

shortening or contraction of the opposite side. We may now consider the evidence as to whether or not the turning away is actually due to a lengthening of the side stimulated.

Very little evidence can be obtained regarding this from observation of the normal moving animal, because the general appearance in the turning would be the same whether it were due to a shortening of one side or a lengthening of the other. The results from certain sorts of operation, however, give definite evidence on the question.

A specimen split longitudinally in the posterior end, as shown in Fig. 17, *a*, and the cut was extended forward to the posterior border of the head region. Several days were

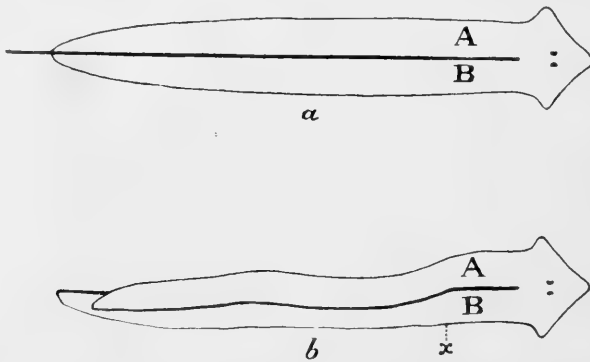


FIG. 17.—*a*. Operation diagram. *b*. Showing side A supported on B. For further explanation see text. (The pharynx is omitted for the sake of clearness.)

allowed for recovery from the shock of the operation, care being taken to prevent the two parts from growing together again. By this time the cut edges had healed well, and the specimen was in good condition for experimentation. The results of mechanical stimulation were as follows: strong stimulation of the head or anterior part of the body on either side caused the negative reaction; the anterior end turned away from the stimulus. But it was possible to tell in this case which of the two pieces or halves of the body were effective in producing the turning. It could be seen clearly that the half stimulated, immediately on stimulation, flattened

out slightly ventrally, thus bringing the ventral cilia in close contact with the bottom, as is necessary for their effective working. At the same time it lengthened along its outer side, thus forcing the anterior end around towards the side opposite from the stimulus. That the "side opposite" had nothing to do with the turning could be observed in many cases directly, for this side (B) would remain in an almost entirely relaxed condition after the stimulus was given, and not get any effective hold on the bottom so that it could affect the movement. It was further possible by a little manipulation to get the piece B laid over on A so as to be practically entirely supported by it, as shown in Fig. 17, *b*. If with such conditions the worm was stimulated rather strongly on the A side of the head, it gave a strong negative reaction, the point about which the turn was made being as far back as *x*. Evidently with part B up on the dorsal surface of A, and consequently having no hold on the bottom, it could have no effect in the reaction. The reaction must have been due to the side A alone. The same thing could be shown by very gently lifting on a needle the side B so that it was not in contact with the bottom, and then stimulating A, when again the negative reaction occurred. This experiment I have repeated with variations many times, but always with the same result, showing that the side stimulated is the effective one in producing the turning.

It may be mentioned here that the effect of strongly stimulating the posterior end of either of the two pieces of a specimen slit in this way was to cause a local contraction of the piece stimulated, and a crawling movement of the short portion of the body in front of the slit. This crawling was not very effective, since so small a portion took part in it, but it is of interest to note that what crawling appeared involved only the uncut part of the body.

It being established that the side stimulated produces the turning, the question may be raised, how, supposing in these longitudinally split individuals that this side does produce the reaction, is it known that it does this by lengthening

along its outer margin rather than by actively contracting on its inner cut margin? This question may be answered by operative experiments of a different character. If the side stimulated, acting independently, produces the reaction by lengthening on its own outer side, then an isolated longitudinal half of the body ought to be able to give only one reaction wherever stimulated, or, in other words, it ought always to turn towards the same side. Furthermore, such a piece ought always to turn towards the cut edge, since only on the side opposite to this has it a margin possessing the necessary circular muscles for extension (*vide sup.*, pp. 556, 557). On the other hand, if the contrary view is correct, that the turning away is due to contraction of the longitudinal muscles on the side opposite that stimulated,



FIG. 18.—Showing the appearance of a longitudinal half of a planarian when at rest.

then such an isolated longitudinal half of the body ought to be able to turn either way, according to the localisation of the stimulus, since there are longitudinal muscle-fibres along the cut edge as well as along the other. We may determine from experiments which of these two views is correct.

Unfortunately, it is impossible to get any clear evidence on this point from entirely separated longitudinal halves of the worm. When a planarian is split in two lengthwise each of the pieces immediately becomes strongly contracted longitudinally on the cut side, the apparent purpose of this reaction being to reduce the exposed surface at once to a minimum. After this strong contraction has taken place, giving the piece the form shown in Fig. 18, no further progressive movement can take place, and the general tonus

becomes immediately very much lowered. In view of these facts it is impossible to get any very trustworthy results from the stimulation of such a piece.

There is another operation, however, which, while it does not isolate completely two longitudinal halves of the body, yet does separate into longitudinal halves the essential reacting parts, namely, the head regions. This is the splitting of the worm in the middle line for a short distance back from the anterior end, as shown in Fig. 12. After this operation the two anterior pieces move about violently and independently for a time, taking all the various positions shown in Fig. 19. The animal soon recovers from the imme-

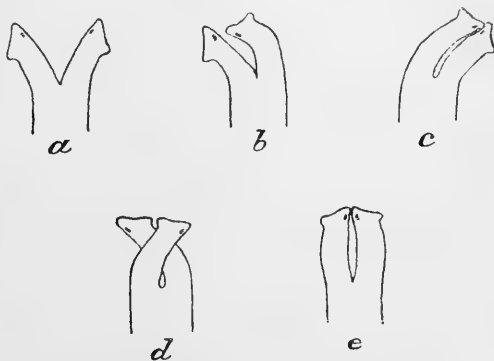


FIG. 19.—Diagram showing the different positions taken by the two components resulting from longitudinal splitting of the head.

diate effects of the operation, glides about in a normal way, only at a rather slow rate, and responds well to stimuli. The anterior piece keeps comparatively straight, there being much less tendency to contraction on the cut side than when the split extends the whole length of the body. The reactions of such a specimen to mechanical stimuli are as follows. To stimuli applied at the posterior end along the sides of the body the reactions are precisely the same as those already described for the normal individual. Stimulation in the regions *aa* (Fig. 20) of moderate or strong intensity produces the negative reaction. The organism turns away from the

side stimulated quite as promptly and in the same way as does a normal specimen. If now the cut edges A and B (Fig. 21¹) are stimulated in the same way (a needle may best be used for this) the specimen will always turn towards the stimulus. This can best be brought out by describing a typical case in which a series of fifty stimulations in the regions A and B were made on a favourable individual cut in this way. In thirty-nine of the reactions the animal turned towards the stimulated side. That is, if the stimulus was applied at A the animal turned in the direction of the arrow *a*; while if B was the stimulated edge the reaction was in the direction of the arrow *b*. In eight of the remaining eleven trials the reaction was indifferent. The animal stopped at

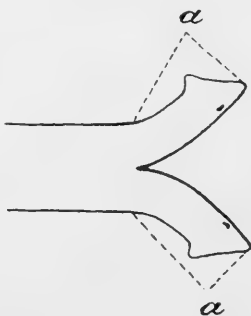


FIG. 20.—Operation diagram. See text.

stimulation and then started moving straight ahead again, the stimulus evidently having been ineffective so far as special reaction is concerned. In only three cases out of fifty did the specimen turn away from the stimulus. Since it required the greatest care in manipulation to give the stimulus to one cut edge without touching the other side, especially in view of the fact that the animal was moving all the time, it seems very probable that in these three cases a stimulus was accidentally given to the side which it was not intended to stimulate. The same general result of turning

¹ After this operation the two parts of the head usually take the position shown in this figure after the first spasmodic movements following the operation have ceased.

towards the stimulus when applied to the cut edge was obtained in several other series with this same specimen, and many times with other specimens similarly mutilated. It will be seen that this is the result which would be expected if the turning away is due to lengthening of the side stimulated. Stimulation of either side of the cut portions, inner or outer, causes turning in the same direction, and that

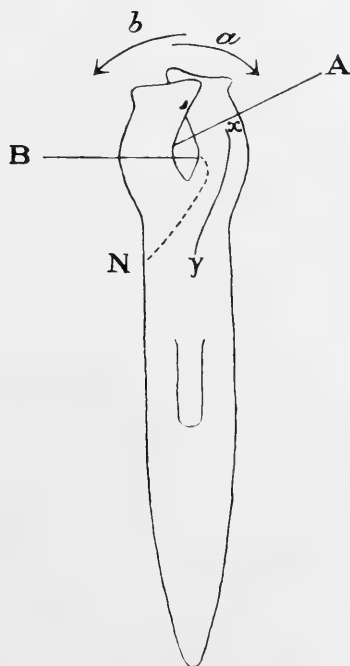


FIG. 21.—Diagram to show the reactions to mechanical stimuli and their mechanisms in the case of a specimen in which the head has been split longitudinally. For further explanation see text.

direction is the one in which turning would be caused provided each piece did actively lengthen on its outer side. There seems to be no reason whatever, if the turning away were due to contraction of the side opposite that stimulated, why the specimen should not turn away from stimuli applied to the cut inner edges. This it does not do. There seems to be no escape, then, from the conclusion that the turning

away from the stimulus (negative reaction) is due to a lengthening of the side stimulated.

It may possibly be objected to the last experiment that the impulse from a stimulation at, for example, B (Fig. 21) took the path indicated by the dotted line in that figure, and caused a contraction on the left side of the body, so that really the observed turning was the result of a contraction on the side opposite that stimulated. To this objection it may be answered that by stimulating different points along the edge B it is possible to cause the point about which the turn occurs as a pivot to be located anywhere along the line xy . It is very evident that contraction of muscles in the region N can have nothing whatever to do with turning of the right piece about the point x . So this objection is without force.

As the process of regeneration of a cut longitudinal half of the body goes on, the piece will straighten out from the curved form it takes after the cut is made, and it is consequently possible to obtain specimens in which the regeneration of the missing half of the body has produced only a very small amount of new tissue, and which are at the same time nearly straight in outline and able to make progressive movements. The reactions of such partially regenerated specimens are of importance as throwing light on the normal mechanism of the reactions. The reactions of a typical specimen of this sort may be described in detail. On October 10th, 1901, a small piece of the anterior end of a specimen of *P. maculata* was isolated. The piece was cut as nearly as possible in the form shown in Fig. 22, *a*. On October 16th the piece had the form shown in Fig. 22, *b*. A narrow strip of new tissue had formed down the right side, and the formation of the outline of the head and of the right eye was just beginning. At this time the reactions of the specimen were as follows. Stimuli applied at y caused the head to turn sharply away from the stimulus (typical negative reaction). This reaction was quite like that given by a normal individual stimulated in the same way. Stimulation at x , however, produced no trace whatever of a negative reaction. On

stimulation at this point the specimen contracted longitudinally, and then started moving ahead again in exactly the same direction in which it was going before stimulation. It was impossible to induce any turning away following stimulation of the side *x*, although this was tried many times.

Now it is evident that this specimen comes very near to being an isolated longitudinal half-planarian. All the structures of the original one half are present, and there is only a very little of the other side of the body produced in the line of new tissue, down the originally cut edge. In this new tissue there is probably very little differentiation, and the muscle layers are not well formed. It was brought out above (p. 610) that an isolated half of the body ought to be able to

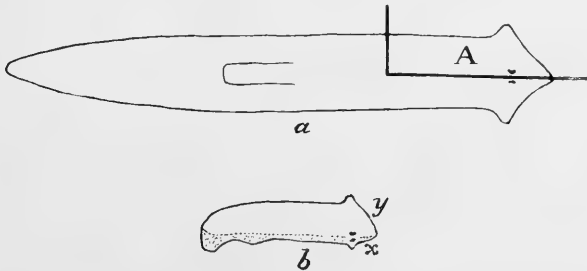


FIG. 22.—*a*. Operation diagram. *b*. Piece which regenerated from A in Diagram *a*. The new tissue is indicated by stippling.

give only one reaction, or, in other words, ought to be able to turn the body in only one direction in response to stimulation, provided this turning is due to an extension of the stimulated side. We find precisely this result in the regenerating specimen just discussed. It turns away from stimuli applied at *y* because on that side are present all the muscles necessary for extension just as in a normal animal. It does not turn away from stimulation of the side *x* because it has not the necessary muscles for extension on that side. On the view that the turning away is due to contraction on the side opposite that stimulated, there is no reason why stimulation at *x* should not cause the animal to turn away from the stimulus, because the opposite side (*y*) has all its muscular mechanisms intact.

The reason why the specimen in this last experiment does not turn towards the stimulus when stimulated on the side *a*, is apparently because the regeneration has proceeded only far enough to produce just enough new tissue to form the beginning of a new side to the body. This new side receives the stimulus and is sufficiently potent to determine the reaction of the whole (the straight longitudinal contraction), but is lacking in the mechanism necessary to produce its own proper reaction, the negative reaction. On the other hand, in the case of the individual with the split anterior end, each piece turns towards the stimulus after stimulation of the cut edge because here only one half the organism is present either to be stimulated or to react; there is not even the beginning of the formation of a new side along the cut edge.

Putting all the evidence together, I think it must be regarded as demonstrated that the turning away from the stimulus in the negative reaction to mechanical stimuli is due to an extension of the side of the body stimulated. This extension is brought about by the contraction of the circular and dorso-ventral muscle-fibres—probably also assisted by the transverse and oblique systems of fibres—in the region stimulated. This reaction is a simple reflex act involving only the side stimulated. The normal organism, so far as this response is concerned, is to be considered as composed of two identical, but in a certain sense independent longitudinal halves. Thus, representing these halves diagrammatically, as in Fig. 23, *a*, the evidence presented indicates that stimulation of one side of the worm, as *A*, causes a reaction in that side, and, so far as essential features of the directive reactions go, only in that side. The movements of half *A* after its stimulation determine and, in fact, cause the reaction of the whole animal. Furthermore, these longitudinal halves retain their individuality as halves if they are isolated from each other. A separated half-worm (longitudinal) reacts as a half-worm, just as it did when in connection with the other half in the body, and not, as might perhaps be expected on a priori grounds, as a whole worm. It reacts as a whole

worm only after a new half has been regenerated along its cut edge. The various stages in the change from the reactions as a half-worm to those as a whole worm can be followed step by step as regeneration proceeds. The new tissue formed along the cut edge very quickly takes on some of the functions of a side. When only a narrow strip has been formed it serves for the reception of the stimulus, and hence stops the reaction of the opposite side, as in the experiment last discussed. To make the meaning more clear, reference may be made to diagrams *b* and *c* of Fig. 23. In *b* is represented, in a straightened position, the half B of a normal worm

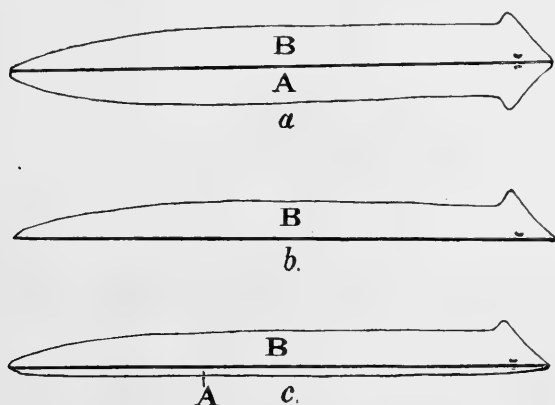


FIG. 23.—Diagrams to show the relations of the halves of the body of *Planaria* to the reception of stimuli, and the reactions thereto. See account in text. (The pharynx is omitted for the sake of clearness.)

immediately after being separated from the other half, while *c* represents the same half after regeneration has begun and a strip of new tissue has been formed down the cut edge. Now stimulation of the cut edge of *b* causes the anterior end of the piece to turn towards the stimulus, i. e. to give its own proper negative reaction (cf. experiment given above on slitting anterior end). This is because in this case it is side B that is stimulated, although along its inner edge. Stimulation along the right-hand edge of *c* does not cause the turning towards the stimulus, because in order that this

may take place it would be necessary for the side B to give its proper negative reaction. It cannot do this because it is not directly stimulated, but the new very small side A is stimulated. This side may not have the necessary muscles to give a negative reaction itself—as in the experiment described above,—yet may receive the stimulus and so indirectly prevent B from reacting. Another way of expressing this same fact is by saying that in regenerating longitudinal halves of planarians the physiological middle line remains at the line of the former cut edge for some time after regeneration has begun.¹ In connection with this discussion of the reactions of half-animals it is greatly to be regretted that Willey ('97) did not get any data on the reactions of the remarkable form *Heteroplana*. In this form we have a natural "half-planarian," or very nearly that. One side is so greatly atrophied as to be practically absent. It seems to me very probable that this organism would react to stimuli in much the same way that a longitudinally split specimen of *Planaria*, which had begun to regenerate, does.

I do not wish it to be understood from the analysis of the negative reaction which has been given that I intend to maintain that in this reaction the side opposite that stimulated never contracts longitudinally. It probably often does this, especially in cases of very strong stimulation which cause a general excitation and reaction of the whole body. I have merely wished to show that the fundamental basis of the negative reaction is the extension of the side stimulated. It seems to me quite possible that it may be shown by close analysis in other cases that supposedly crossed reflexes are not fundamentally such at all.

We may now pass to a brief consideration of the mechanism of the positive reaction of the planarian to mechanical

¹ I have records in my notes of experiments which show that in the case of oblique cuts the physiological middle line remains at the cut edge until after the new head is well formed in the new tissue on the oblique edge. Lack of space forbids detailed description of these experiments here.

stimuli. As has been shown above, removal of the anterior end of the body containing the brain causes the disappearance of this positive reaction, and this result is probably due rather to the lowering of tonus than to the removal of any special centre having the causation of this reaction as its function. Additional evidence on this view that lowering of the tonus is the chief cause of the disappearance of the reaction is found in the fact that other injuries to the head, such as longitudinal splitting, which produce a lowering of the general tonus, also cause the disappearance of the positive reaction.

This very close dependence of the reaction on the general tonic conditions of the organism makes its analysis difficult, but it seems most probable that its mechanism is as follows:—a light stimulus, when the organism is in a certain definite tonic condition, sets off a reaction involving (1) an equal bilateral contraction of the circular musculature, producing the extension of the body; (2) a contraction of the longitudinal musculature of the side stimulated, producing the turning towards the stimulus (this the definitive part of the reaction); and (3) contraction of the dorsal longitudinal musculature, producing the raising of the anterior end. In this reaction the sides do not act independently, but there is a delicately balanced and finely co-ordinated reaction of the organism as a whole, depending for its existence on an entirely normal physiological condition. It is to be noted, however, that the definitive part of the reaction, namely, the turning, is a response of the side of the body stimulated. This point is one of fundamental importance for the general theory of the reactions.

The mechanism of the other reactions to mechanical stimuli are evidently very simple. The crawling movement, which must be considered as the specific reaction to mechanical stimulation of the posterior region of the body, is due to rhythmical contraction of the longitudinal musculature. The only other reactions to mechanical stimulation are local contractions, whose mechanism is evident.

e. Features in the General Behaviour of the

Organism which the Reactions to Mechanical Stimuli explain.—That much of the behaviour of planarians in their natural surroundings is the result of the reactions above described is very evident to any one watching them. Among specific features of this sort in which these reactions play a part may be mentioned the escape from enemies or harmful surroundings, the getting of food (to be discussed in detail later), the localities chosen for coming to rest, the behaviour on meeting solid obstacles in the path of movement, the passing on to the surface film, etc. All of these need not be discussed specifically, as their relations will be evident enough on a moment's thought, but the last two deserve special mention.

The behaviour of planarians on meeting solid bodies in their path in the course of movement is entirely made up of reactions to mechanical stimuli. The behaviour in detail is as follows :—If a gliding specimen meets squarely head-on an obstruction of considerable size, so that it cannot glide over it without changing to some extent the position of its long axis, it will stop an instant, raise the head, let it drop down till it touches the obstruction again, and then glide directly up on to and over the solid body. This behaviour is invariable, so far as my observations go, if the worm meets the obstruction squarely. It is at once seen to be merely a special case of the usual reaction to a weak mechanical stimulus, characterised by the raising of the head. The behaviour is evidently purposeful in the long run, because it will take the organism up on to food material just as well as indifferent bodies. If the gliding worm meets the obstruction obliquely the behaviour depends in large part on the physical nature of the object. If it is food material, or something else of a rather soft and yielding texture—as, for example, another planarian,—the worm will immediately raise the head, turn it towards the object, and crawl up over it. This behaviour is evidently the typical positive reaction to a weak mechanical stimulus. A special and rather curious case of this positive reaction, which I have twice observed,

appeared when two specimens gliding along, with the anterior ends slightly raised in the normal manner, met head-on. Both were simultaneously stimulated to the positive reaction and raised the anterior ends, and then let them drop again. As they came down the two ventral surfaces were brought squarely together in the way shown in Fig. 24; then each started gliding up the ventral surface of the other. In a movement as a result of the constantly changing form of the body, the ventral surfaces slipped off from one another and the two worms went on their way. When the obstruction is a hard body, as a piece of glass, the specimen meeting it obliquely usually turns the head away slightly at the first contact (negative reaction), and then glides along parallel to the edge of the body for a distance. If it happens to touch it again with the side of the head, it frequently gives the negative reaction and turns away again. After the solid body

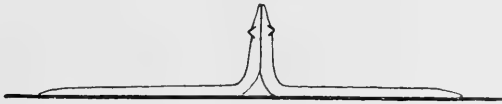


FIG. 24.—Side view of two planarians starting to glide up on the ventral surfaces of each other.

has been touched several times, however, the positive reaction is usually given, and the worm passes at once up on to the solid body. This behaviour is shown in Fig. 25. The precise form of the behaviour on meeting obliquely a solid body in the path varies considerably with the general physiological condition of the individual. In case it is much excited, the first touch will induce a strong negative reaction, and the individual will turn away and pass out of the neighbourhood. In the cases where the final positive reaction is preceded by two or three negative ones, it would seem as if repetition of what must be an almost identical stimulus causes it to become in effect weaker. Leaving aside all variations in the exact character of the behaviour on meeting a solid, the important point to be brought out is that all this behaviour is based on the simple reactions to mechanical stimuli. The

exact behaviour in any given case depends on several different factors. These are the position of the animal with reference to the obstruction, the physical nature of the obstruction, and the physiological condition, whether of greater or less excitation.

So, again, with reference to the habit of the animal of moving about on the surface film, a problem is presented. When a specimen, gliding up the side of a dish, touches its anterior end to the surface film at the point where the latter joins the glass, it immediately gives a characteristic positive reaction, precisely like that in response to any other weak mechanical stimulus. The head is raised and turned towards the side from which the stimulus came, and then dropped

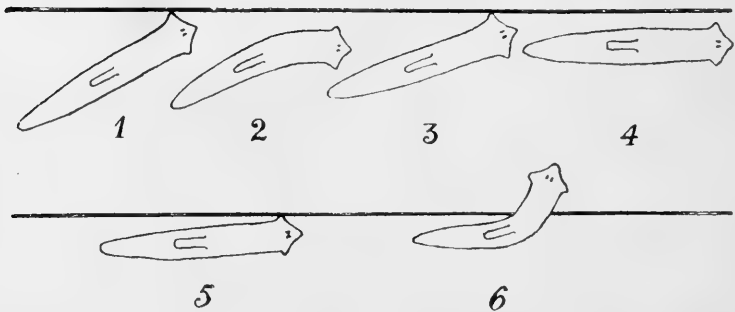


FIG. 25.—1, 2, 3, 4, 5, and 6 are successive stages in the reactions of *Planaria* on meeting obliquely an obstacle in its path. The heavy straight line represents the obstacle.

again. As a consequence of this reaction, the head end comes to rest on the under side of the surface film at a point some little distance out from the side of the dish. The ventral surface of the anterior end of the body flattens out on the surface film, and the animal glides out on to the film, following the direction determined by the reaction of the anterior end. Thus it is seen that the going on to the surface film is only a special case of a response to a weak mechanical stimulus, i. e. the positive reaction, the film itself acting as the stimulant. The leaving of the surface film and passing down the side of the dish is evidently also due to the same positive reaction.

There are a number of other points in the general behaviour which are directly related to the reactions to mechanical stimuli, which will be taken up later in connection with the other reactions.

f. Summary.—Before passing on to a discussion of the next subject, it may be well to summarise briefly the chief findings with reference to the effect of mechanical stimuli on planarians.

It has been shown that the planarian responds in a well-nigh perfect manner to the localisation and intensity of mechanical stimuli. It turns away from strong stimuli (in the long run harmful) applied to the side of the body; turns towards weak stimuli (in the long run beneficial, almost never harmful); it crawls rapidly away from strong stimuli applied to the posterior end; backs and turns away from similar strong stimuli applied at the anterior end.

It has been shown, further, that these reactions have all the characteristics of reflex actions, complex, it is true, but still reflexes.

The mechanisms of the reactions to unilateral stimulation are unilateral, and lie in the side stimulated.

Discussion of the implications of these results on mechanical stimulation, with reference to the psychology of the organism and the general theories regarding the reactions of organisms to stimuli, is deferred till the results from other sorts of stimuli are in hand.

II. Reactions to Food and Chemical Stimuli.

Evidently one of the most important factors in the sum total of the activities of any aquatic organism is its reactions to chemical substances. Its ability to receive chemical stimuli and react to them must be of prime importance in its struggle for existence, for in its natural habitat such an aquatic organism must be almost constantly encountering different chemical substances. Some of these may be harmful and some beneficial, and it would seem that if a species is

to survive, its individuals must have some sort of reaction whereby they may avoid the harmful and take advantage of the beneficial. In the case of planarians, the reactions to chemicals seem to be of about equal importance with the reactions to contact stimuli in the general activities. Since the reactions to food substances are a special case of the reactions to chemicals in general, they may be discussed first.

a. Food Reactions.—The nature of the things used as food by fresh-water planarians has been discussed already in the section on "Natural History," and hence need not detain us here.

A typical case of the food reactions to a bit of crushed

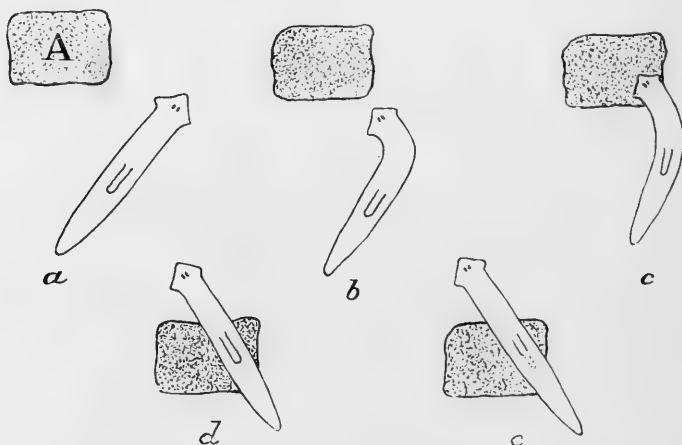


FIG. 26.—Diagram showing the successive stages in the normal food reaction of *Planaria*. A represents a small bit of meat.

mollusc may first be described, to serve as a basis for the account.¹ If a piece of the body of *Physa* which has just been extracted from the shell and crushed between the points of a pair of forceps is placed in a small dish containing a number of active planarians, it will result from chance alone that some of the flat-worms must in course of time pass near the food material. For a very short time after the food has

¹ The food reactions of *Planaria* have been briefly described by Bardeen (:01, *a*).

been placed in the dish specimens may pass very near it—within two or three millimetres—without being affected in any way. They simply glide straight by as if there were no food there. After a few minutes have passed, however, it will be found that a worm coming near the food is affected in a very characteristic manner. Its behaviour is as follows:—When within about three or four millimetres of the piece of meat (Fig. 26, *a*) it stops abruptly, raises the head, and turns it towards the food (Fig. 26, *b*). As the head is raised and turned the gliding is resumed, and the head being almost immediately lowered, the movement is directly towards the food. Thus far the reaction is evidently precisely like the positive reaction to weak mechanical stimuli, and so we may speak of it as the positive reaction to food, the reaction being the same in the two cases, though the stimulus differs. When the anterior end of the head



FIG. 27.—Diagrammatic side view of *Planaria* to show the "gripping" of a bit of food, A.

touches the food it flattens down upon it, and, if the configuration is such as to make it possible, "grips" it (Fig. 26, *c*). The details of this "gripping" (shown in side view in Fig. 27) are as follows:—The anterior end closes down over the very edge of the piece of food, or over the whole piece provided it is small enough, and then apparently squeezes it by contraction of the longitudinal muscles on the ventral surface of the head. The action is very characteristic, and evidently forms an integral part of the normal food reaction. Its probable function will be brought out later. While it is taking place the worm as a whole stops its progressive movement and remains quiet. After the "gripping" has continued for some time the worm starts gliding ahead up on to the food. It passes forward till the point where the opening for the extrusion of the pharynx is located is approximately over the place pre-

viciously "gripped" (Fig. 26, *d*). Then the pharynx is extruded and feeding begins (Fig. 26, *e*). After a time the worm voluntarily leaves the food and glides off over the bottom.

Having described the typical case of a food reaction, we may take up some of the more important variations from the type, and describe the various phases in the reaction in greater detail.

Starting with the very beginning of the reaction, it may be said that the distance from the food at which any effect on the planarian is produced varies greatly, as is to be expected. This distance, of course, depends on the extent which the juices or chemicals of the food have diffused from it. When a piece of meat is first put into the water specimens will pass very close to it without being stimulated. In fact, if a specimen finds a piece of food within three or four minutes after it is put into the dish, it will usually have done so as a result of accidentally coming in contact with it. As has been brought out above, when a gliding worm touches anything of a rather yielding texture, like food, it immediately gives the positive reaction and passes up over it. This plays an important part in the getting of food, because, as I have found in experiments, unless the food is crushed and pressed with forceps the juices diffuse rather slowly, and for some time specimens will not give the positive reaction unless they actually touch the food. On the other hand, after the food has been in the water for some time, so that diffusion has taken place, the distance at which specimens may be affected becomes quite considerable. I have seen specimens gliding by a small piece of meat at a distance of $1\frac{1}{2}$ cm. from it give the positive reaction and turn towards it. At greater distances than this food is not effective, according to my observations. The distance from food at which a given specimen will give the positive reaction and go towards it depends also on the physiological condition of the individual. Specimens in a state of general excitation will, as I have frequently observed, go closely by

a piece of food without turning towards it, while other specimens in a more normal condition will give the positive reaction some distance from it.

After the first specimen has begun feeding on a piece of material the zone of influence of that piece becomes almost immediately widened appreciably. As the number of feeding specimens increases the area in the surrounding water which affects others becomes correspondingly greater. This phenomenon is very striking in many cases, as an illustration will indicate. Several pieces of crushed snail were put in a dish with a number of planarians. In a short time a specimen in gliding about the dish had come near to one of these pieces, had given the positive reaction and begun feeding. At almost the same time another of the pieces of food had "attracted" another specimen. The other bits of food were quite similar in every way to these two, and lay in the dish not far from them. Yet at the end of fifteen minutes the two pieces by which the first two worms had been affected were completely covered with feeding specimens, while the remaining pieces of food, with a single exception,¹ did not have a specimen on them. This increase in the effectiveness of the food as a stimulus must be due to the diffusion of more chemical substance from it. Apparently the increase is due either to some secretion of the feeding animals or to some change which they induce in the food. It is probably due to a combination of these two factors. That a digestive secretion is poured out through the pharynx of the feeding worm is well known, and clearly shown by the appearance of a piece of food on which a specimen has been feeding. The surface of the meat is turned white, and rendered very soft and almost flocculent. It is probable that this digestive secretion acts as a positive chemotactic stimulus to other worms, and that coupled with this there is an increased diffusion of juices from the food itself caused by the changes which it is undergoing.

The reaction which is caused by this chemical stimulus

¹ One piece farthest removed from the others had a single specimen on it.

from the food is evidently essentially the same thing as the positive reaction given to weak mechanical stimuli. It consists in a turning of the anterior end of the body towards the source of the stimulus. There is no reason for supposing that its mechanism is in any way different from that of the same reaction to mechanical stimuli, and hence this need not be further discussed here. A question of prime importance with regard to this positive reaction in response to chemical stimuli, which was not taken up before, is—how well localised, with reference to the stimulus, is the reaction? or, in other words, how precisely does the anterior end point towards the source of the stimulus,—in this case food? Have we here a clear-cut orienting response? In answer to this problem it may be said that when the worm is only a short distance from the food the response is very precise. The anterior end is brought by the first positive reaction so as to point exactly towards the meat, and as the worm glides ahead it never misses it. This is true where the specimen is near enough (usually within three quarters of its own length), so that the stimulus which reaches it is a fairly strong one. In case the worm is stimulated near the edge of a large diffusion area when the stimulus is very weak, the first reaction may not suffice to direct the animal straight towards the food. In this case the behaviour is usually like that shown in Fig. 28, in which the line B, B, B, represents the effective margin of the diffusion area of the piece of food A. (By “effective margin” is meant the line outside of which no effect is produced by the food on passing specimens.) The first reaction which the worm gives on reaching this diffusion area (Fig. 28, 1 and 2) is a weak positive one. It then proceeds on the new path into this area, but not directly towards the food. After a short time, however (Fig. 28, 3), it is again stimulated to a positive reaction (4). This time both the stimulus and the reaction are stronger than before, and the worm is directed more nearly towards the centre of diffusion, but still not exactly. When it gets opposite the food again (5) another positive reaction (6) is given, and this

time, since the stimulus is a rather strong one, the reaction is a very precise one, and the subsequent movement carries the animal directly to the food (Fig. 28, 7). This behaviour is typical for this sort of stimulation, but may vary in its component phases, depending on the relative strength of the stimulus—the distance from the food when first stimulated.

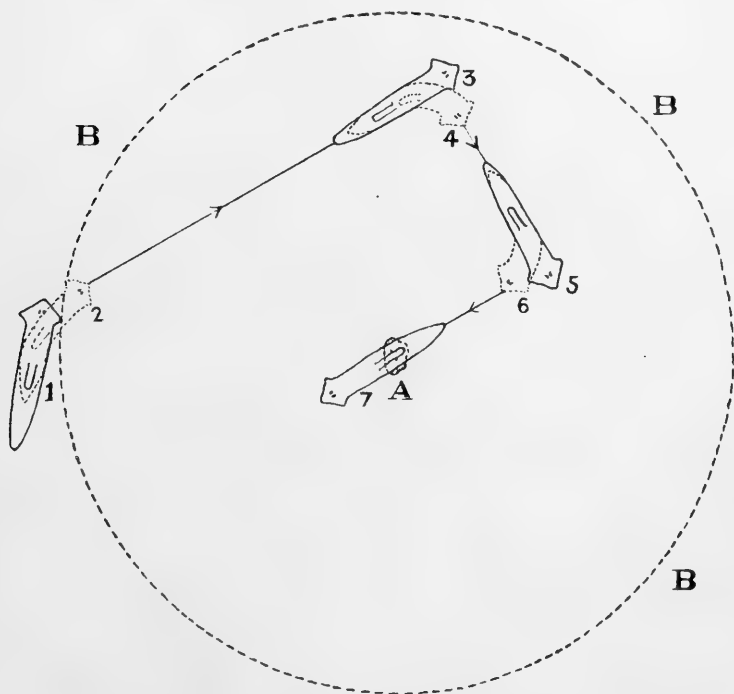


FIG. 28.—Diagram showing the reactions of *Planaria* to food (A) from which juices have been diffusing into the water for some time. B, B, B, represent the effective margin of the diffusion area of the food A. 1, 2, 3, 4, 5, 6, and 7 are successive positions taken by the organism.

Thus either two or as many as four positive reactions may be necessary to bring the animal to the food. This shows clearly that with reference to chemical stimuli, the precision of localisation of the positive reaction decreases as the intensity of the stimulus diminishes. Indeed, I have observed what is evidently an unlocalised positive reaction, although

this seems paradoxical. The behaviour was as follows:—A large diffusion area had been formed, and a specimen was stimulated to a weak positive reaction at a distance of about twice its own length from the food (Fig. 29, 1). It passed into the diffusion area, but did not give another positive reac-

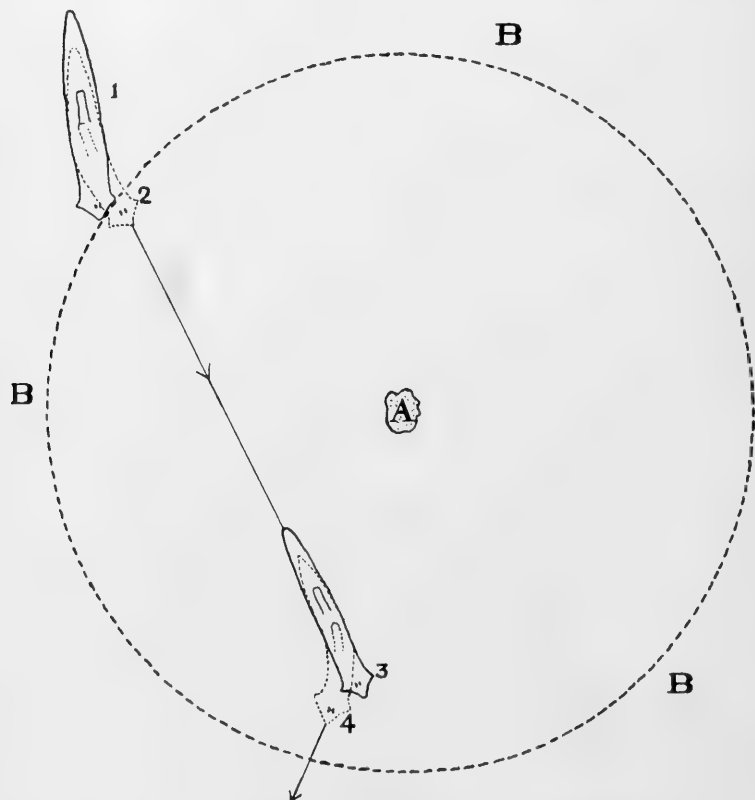


FIG. 29.—Showing the reaction of a planarian to a very weak food stimulus. Letters as in Fig. 28.

tion when opposite the food, but instead glided by and away from it. When it had gone some distance in this direction it stopped and gave a very clear and characteristic positive reaction, so far as the form of the reaction indicated, but with the turn away from instead of towards the centre of

diffusion. There was no doubt of the character of the reaction; the head was raised and the body turned in the usual manner of the positive reaction, which one can never mistake after once having become familiar with it. The specimen kept on in the path determined by this last reaction (Fig. 29, 4), and passed entirely out of the region of the food. Evidently in this the worm was stimulated very weakly by a chemical, and the stimulus was nearly as strong on one side of the body as on the other, and when the reflex was set off it was on the wrong side of the body. This is not the usual result of weak stimulation, and has been observed in only two cases, but it serves very well to show the decrease of the power of localisation when the stimulus is very weak.

When, as frequently happens, the worm approaches the food exactly head-on, the reaction usually consists merely of that portion of the reflex expressed in the raising of the head, while the worm keeps on in its straight path till it reaches the food. The head may be waved from side to side slightly, but the general direction of motion is not changed. The action evidently corresponds to the positive reaction following weak mechanical stimulation of the dorsal surface of the head in the middle line, as described above. In some cases, however, I have observed very active and hungry specimens of *Dendrocœlum*, sp., which were going straight towards the food, give a complete positive reaction and turn to one side and start off in a new direction away from the food. This, however, of course brought the specimen at once into a position where the stimulus was acting unilaterally, and it again gave a positive reaction, this time heading it again for the food, which it usually reached without further reaction. But in some cases I have observed the specimen give so strong a reaction as to be taken almost directly away from the food by the subsequent movement, and, passing out of the area of diffusion, fail to reach it at all. Specimens behaving in this way were "wild" in their general reactions. The responses were very vigorous, but

not localised with reference to the stimulus with the usual precision.

The "gripping" of the food substance by the anterior part of the worm is a very characteristic feature of the normal food reaction. Its exact form depends on the configuration of the food or other body "gripped." In its most typical form, where the food material is in the form of a cylinder, or approximately such, the action reminds one of the action of the human hand in grasping a stick. The tip of the head closes over the material in the same way that the fingers do, while the region just behind the auricles bears the same relation as does the proximal part of the palm, just in front of the wrist, in grasping. After the head has been placed over the material in this way it can be seen to contract rather strongly, and thus literally squeeze the food. In case the surface contour of the food does not admit of this reflex being carried out in its typical form, as close an approximation to this is made as possible. To compare again with the human hand, when the surface is flat, or forms the surface of a cylinder of large radius, the ventral surface of the head is pressed closely to it, the tip attempting to dip in, as it were, below the surface, in just the same way that a man "claws" with his finger tips in attempting to obtain a hold on a similarly configured body, too large for complete grasping.

While the "gripping" is in general a very characteristic feature of the food reaction, it may be omitted in rather exceptional cases. The cause for the omission where it occurs, or any laws governing the matter, I have not been able to discover. A necessary accompaniment of the "gripping" of the food is the cessation of the forward movement of the animal as a whole. This pause when the food is first touched by the anterior end and before the worm passes up on to it; occurs in practically every case, whether the gripping accompanies it or not. The length of the pause is, of course, considerably greater when the "gripping" occurs than when it is absent.

The function of the "gripping" of the food material before

feeding begins is not immediately apparent, but I am inclined to think its purpose is to intimately test the substance with regard to its availability as food. Some evidence on this point and further discussion regarding it will be introduced later.

After the preliminary pause and "gripping" of the food the worm glides up on to it to begin active feeding. The position taken by the worm brings out a very nice correlation in reflexes. In a very large number of cases (certainly over 75 per cent., so far as my observations have gone) the worm advances over the food until the pharyngeal opening is exactly over the place where the first "gripping" occurred, and there the pharynx is extruded and feeding begins.

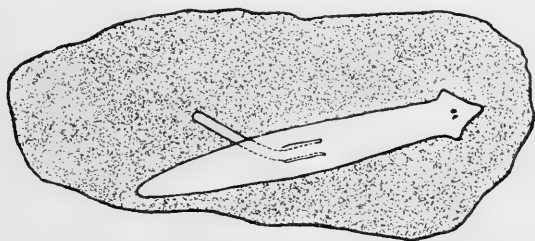


FIG. 30.—Diagram showing great extension of the pharynx. The stippled area represents food substance on which the planarian is resting.

When the worm reaches this position the posterior part of the body relaxes and takes on the appearance characteristic of the resting specimen. The pharynx is thrust out, and becomes attached very quickly. As it passes out through the opening in the body-wall it becomes usually considerably extended, and its diameter becomes correspondingly smaller than when it is in the pharyngeal sac. It may or may not attach to the food directly beneath the body. When conditions are favourable it usually does, and consequently cannot be seen on looking down on the animal from above. On the other hand, I have frequently seen it stretched out and attached some little distance to one side of the body, as shown in Fig. 30. The stimulus, causing the extrusion of the

pharynx, is the contact of food or other solid body with the pharyngeal region of the ventral surface, together with an appropriate chemical stimulus. The pharynx is not extruded until the animal gets up on to the food so that the opening of the pharyngeal sac is in direct contact with it. This can be demonstrated by direct observation by the use of a very small piece of food material and a plane mirror placed beneath the glass dish in which the specimen is moving. By lifting gently the posterior end of the body on a needle it can also be seen that the pharynx is not extruded before it is over the food. The most striking illustration of the correlation in the reaction which brings about the extrusion of the pharynx when it is just over the food, is to be seen when a specimen of the nemertean *Stichostemma asensoriatum* is used as food, and the long axis of the planarian and of the nemertean are at right angles to each other. After first "gripping"



FIG. 31.—Diagrammatic longitudinal section of a planarian feeding on a nemertean (shown in cross-section at \times).

the nemertean the planarian glides along over it until the pharyngeal opening is just above it, and then pauses, and the pharynx is extruded and attached (*a* and *b*, Fig. 31). These facts strongly indicate that the effective stimulus for pharyngeal extrusion is received, at least in part, in the pharyngeal region itself. That it is necessary for both contact and chemical stimuli to act to produce the extrusion of the pharynx may be shown by experiments on specimens gliding on the surface film ventral side uppermost. If, with such a specimen, a chemical known to produce under other conditions extrusion of the pharynx, is allowed to come in contact with the pharyngeal region, there is no result. Of course in performing this experiment proper precautions were taken not to disturb the animal by allowing the solution to drop upon it. Another demonstration of the same fact that a chemical stimulus alone does not suffice to cause extrusion

of the pharynx is that specimens immersed in favourable solutions, such as sugar solutions, do not show this phenomenon. That mechanical stimulation alone does not suffice is demonstrated by the fact that planarians pass over and rest on other planarians without extruding the pharynx, although the consistency of their bodies is evidently much the same as that of the animals used as food. In fact, they will be used as food frequently if they are wounded so as to afford the proper chemical stimulus. The stimulation of the anterior end of the body by the food seems also to be necessary before pharyngeal extrusion takes place. The data on this point will be presented later in connection with operation experiments.

The appearance of the body on the food is quite characteristic. As mentioned above, when the pharynx is extruded forward, movement stops, and the posterior part of the body becomes more or less relaxed. The anterior third of the body, however, keeps in movement during a considerable part of the time the specimen is feeding. The head is waved about from side to side, and touched to the food or the bottom of the dish here and there. It keeps its characteristic extended form to a greater or less degree. A favourite position is for the anterior third or half of the body to lie on the bottom and move about, while the posterior part lies up on the food. This is the position most frequently seen in specimens feeding on a rather small piece of meat. When the anterior end gets on the bottom it gives every appearance, in many cases, of attempting to glide away, and being only restrained by the attachment of the pharynx to the food. In other cases, however, the anterior end remains quiet. The importance of the attempted movement will be brought out later. As has been mentioned above, the flatworm is able to move off and drag the food still attached to the extruded pharynx along behind it. In the fastening of the food to the body in this case the sticky slime undoubtedly assists the pharynx.

After the food has been softened by the digestive juices, it is taken into the body through the pharynx.

After the worm has been feeding for a certain length of time it will detach the pharynx and spontaneously move off from the food, the pharynx being withdrawn again into its sac. The length of time after the beginning of the feeding at which this takes place varies very greatly in different cases. I have observed a specimen which fed on a piece of mollusc for as long as an hour and thirty minutes, while in other cases the worm may stay on the food only ten minutes, or even less. Judging from the rate at which food is taken up while the animal is feeding during the day, and from the fact that pieces of meat left in the dish overnight are almost entirely consumed by morning, it would appear that much of the time during the night is spent in feeding when any material available for the purpose is at hand. While the anterior end of the feeding worm retains its normal sensitiveness to stimuli, it nevertheless requires considerable stimulation to induce a feeding worm to leave the food. Shaking of the dish, which would ordinarily set all resting specimens into rapid movement, has little or no effect on feeding specimens. If a worm is suddenly pulled off a piece of meat on which it is feeding a very good view of the extruded pharynx may usually be had, as this organ is retracted somewhat slowly when torn from food in this way.

So far as I have been able to discover, the presence of food in the immediate neighbourhood of a resting planarian has no effect upon it. Apparently the stimulus afforded by crushed meat is not sufficiently strong to produce a response from such an individual. The following experiment copied from my notes will show this.

May 14th, 1901, 3.10 p.m.—A piece of freshly crushed snail was placed 1 mm. distant from the anterior end of a resting specimen. No reaction or other effect produced.

3.30 p.m.—Worm in same position as before.

4.5 p.m.—No change. (At this time the worm was accidentally started into movement and the experiment consequently ended.)

This lack of effect of food on resting specimens may be

the reason for the statement of Bardeen (*loc. cit.*, p 522) "that worms which had been kept in pure rain water for a week or two, and were thus in a hungry condition, would remain unmoved by the presence close by their side of a piece of fresh snail, a food much prized by them."

1. Food Reactions of Specimens after Operations.—For the purpose of throwing light on the general mechanism of the food reaction, experiments were tried on specimens cut in different ways. It is unfortunately very different from practical reasons to get many certain results from these experiments. Many of the results are negative, and hence not entirely conclusive. Since, however, some important facts have been brought out by these experiments, they will be described.

The first operation which will be discussed is that of cutting the animal in two transversely. If such a cut is made in the region in front of the pharynx, the anterior resulting piece, after it has recovered somewhat from the shock effect of the operation, will show the following reaction. On coming into the zone of diffusion about a piece of meat it gives the positive reaction just as a normal worm does, and turns towards the food. On reaching the edge of the meat its behaviour is again like that of the normal animal; it stops, usually "grips" the food, and then passes on over it. At this point appears the striking difference between the behaviour of this anterior piece, which, it must be remembered, has no pharynx, and the behaviour of the entire worm. The anterior piece after gripping the food glides up over it, and without the slightest change, even in the rate of gliding, passes down off of it on the other side. There is not the slightest indication of any stopping for the pharynx to be extruded.

If the transverse cut is made farther back, so that the pharynx is included in the anterior piece, this will then behave with reference to food quite as a normal animal does. It will stop on the food and extrude the pharynx.

The posterior pieces resulting from transverse cuts do not

give any definite food reaction, so far as I have been able to ascertain, until they have been regenerated to some considerable extent. Posterior pieces from which only the head has been cut will glide by pieces of snail on which other worms are feeding, without giving the slightest reaction.¹ In experiments so arranged that the gliding posterior piece would just touch with its anterior end the edge of a piece of food, it gave no reaction. This same arrangement with a normal worm practically never fails to call forth the positive reaction and bring the worm up on to the food. Posterior pieces placed gently on pieces of food material do not extrude the pharynx and start feeding, but immediately glide down from it and over the bottom of the dish. These experiments with posterior pieces have been tried many times and under varied conditions, in the hope that some sort of positive results might be obtained, but never with success. This is true for three days after the operation. After a new head has been fairly well formed the animal will react to food again. The behaviour of one of these posterior pieces on touching with the anterior end a piece of food is very strikingly different from that of a normal animal. The cut piece, if it touches with the front or sides of the anterior end the smallest shred of food material, or any other substance, gives a well-marked negative reaction, and goes in a new direction away from the obstruction. It does not, as a rule, crawl up over anything which it meets squarely "head-on," but instead turns away.

Thinking that possibly the pharynx might play a more or less independent part in the normal food reaction, i. e., that it might have a set of reflexes of its own, not determined by the rest of the body, I tried experiments with the isolated pharynx removed entire from the body. Such an isolated pharynx will remain alive for a considerable period, and respond to stimulation. When first removed from the body

¹ Bardeen (:01, a) has shown that if the transverse cut is in the region in front of the eyes the posterior piece (comprising in this case nearly the whole worm) will react normally to food.

it contracts rhythmically in a longitudinal direction for a time, and then comes to rest at about its normal length when in the body. Mechanical stimulation causes merely longitudinal contraction, while the presence of food near it has no effect whatever. Freshly crushed snail meat placed within a millimetre of such an isolated pharynx had no effect upon it in the course of an hour. I have tried laying the isolated pharynx directly on pieces of meat to see if there would be any tendency for the end of the organ to attach itself as it normally does. This was not done, nor was any other definite reaction produced.

These operation experiments show, so far as they go, that—

(1) The presence of the pharynx in the body (i.e., the functional ability to take food) has nothing to do with determining the reaction of the anterior end of the body to food stimuli. The anterior part of the body gives the same reaction to food in every case, without regard to whether so doing actually puts the animal in a position to get food or not. The reaction is only purposive under certain circumstances; when changed conditions make it no longer purposive, no adaptive change in the behaviour of the anterior end occurs. This shows clearly how little basis there is for considering the behaviour towards food as anything of the nature of intelligent behaviour.

(2) The stopping of the worm on the food under normal circumstances is due to the posterior half of the body, not the anterior. The behaviour of the anterior cut piece in gliding directly over the food is what one might be led to expect from the behaviour of the same part of the body under normal circumstances. As described above, it was seen that the anterior end of the normal individual gives every appearance of attempting to continue moving forward while the posterior part is feeding, and is only prevented from doing this by the mechanical hindrance of the attached pharynx. In a sense, we may consider that in a large degree the work of the anterior end of the body with reference to feeding is over when it gets the animal up on to the food.

(3) The reception of the food stimulus is a function of the head. In other words, the head is the only part of the body capable of receiving very weak chemical stimuli.

(4) Decapitated specimens do not extrude the pharynx, so far as my observations go, even though the proper normal stimuli are given the pharyngeal region. Presumably the brain is the necessary organ in this connection, as we have already seen that the sense organs concerned with the act of extrusion are not those of the head, but of the pharyngeal region.

Bardeen (: 01, *a*, p. 178) states that "the simple reflexes of extending the pharynx and of swallowing are preserved after removal of the head. I found, by repeated trials, that one of the headless pieces could usually be made to eat if it was placed on its back on a slide in a small drop of water. Under the conditions mentioned the pharynx is usually protruded, and will engulf bits of food placed in the mouth." Regarding this conclusion, I can only say that in a large number of experiments with decapitated specimens I have never been able to induce extrusion of the pharynx, under conditions approximating as closely as possible to the normal. I do not wish to affirm that the decapitated planarian cannot extrude the pharynx, but merely that it does not when placed in situations which normally produce pharynx extrusion.

(5) The pharynx is not an independent organ in its reactions, since, when separated from the body, it does not react with reference to the localisation of the stimulus, as it does when normally connected with the remainder of the body.¹

2. Summary of Food Reactions.—It is shown above that planarians have a very definite and characteristic set of reactions to food substances which enable them to become aware of the presence of food, and find it. The importance of these reactions in the life of the individual can hardly be over-estimated. While planarians, like many other lower organisms, can live for a considerable time without food, yet in the long run they must, of course, have it. The question

¹ Evidence on this latter point will be brought forward in connection with the reaction to chemicals.

of how a lower organism gets its food, taking advantage of the good and rejecting the bad, and thus apparently choosing one thing from several, is one of the most interesting and important in comparative psychology.

The food reaction of planarians consists of an extremely well co-ordinated set of reflexes, which may be set into action by stimuli of two sorts,—first, chemical; and second, mechanical. Both sorts of stimuli are, of course, given by the food. The first and most important of all the reflexes in the food reaction is the turning of the head towards the source of stimulation, followed by movement in that direction. This is the reaction which enables the animal to find food. Evidently it is the same thing exactly as what has been described as the positive reaction to mechanical stimuli; or, in other words, the positive reaction to mechanical stimuli is only a special case of the general food reaction. Its primary function is evidently the getting of food, whatever the stimulus which calls it forth. The reason for a food response following mechanical stimulation is to be found in the fact that it most frequently happens that many things (e. g., whole animals) which are available for food are not emitting chemical substances into the water in sufficient quantity to cause an effective stimulus. If the planarian did not give a positive reaction after contact with such bodies they would be missed, and no advantage taken of them as food. By reacting positively to weak mechanical stimuli the animal is in a position to take advantage of the presence of food of all sorts, whether it is in condition such as to diffuse chemical substances through the water or not. This fact that the animals react to food substances as a result of mechanical stimulation affords a possible explanation of the “gripping” phase of the general response. The purpose of this “gripping” may be to bring the sense organs of the head, which are sensitive to chemical stimuli, into very close contact with the substance in order to determine whether it possesses the chemical characteristics of food. In other words, this reaction is a “tasting” reaction, which is made necessary by the fact that

the organism turns toward all bodies of a certain physical texture under most circumstances. The active squeezing of the material in the "gripping" undoubtedly helps to press out to the surface any juices which may be in the material.

In closing the section on food reactions it may be well to give a sort of general picture of the whole behaviour of fresh-water planarians towards food. The method by which the planarian finds material suitable for food is as follows:

1. Chemical substances diffusing from food come in contact with the sensitive head region of the planarian; or—

The moving animal touches with the head some soft substance, and as a result of either of these two sorts of stimulation—

2. The organism gives a positive reaction, i.e. turns towards the source of the stimulus. This reaction is very precisely localised in most cases, and is the most essential part of the whole food behaviour. Its mechanism has been previously described (v. sup., p. 619).

3. When the anterior end squarely touches the food as a result of this reaction it typically closes tightly over it, giving what I have called the "gripping" reaction. This reaction is evidently a very much specialised feeling movement for the purpose of closely testing the chemical nature of material. It is produced by a contraction of the ventral longitudinal muscles of the head region. While it is taking place progressive motion ceases.

4. After this pause the worm glides over the piece of food till the opening of the pharyngeal sac lies over or nearly over the place "gripped," and there the posterior part stops and the pharynx is extruded and attached to the food. The factors determining the place where the pharynx shall be extruded are (*a*) the stimulation of the ventral surface of the body in the pharyngeal region of the food (pure reflex factor), and (*b*) the presence of the brain, which probably acts as a co-ordinating centre for this reaction.

5. A digestive fluid is poured out through the pharynx, and the food is partly digested before being taken up.

6. The softened food is taken into the body through the pharynx.

7. The animal spontaneously stops feeding after a certain time.

The question now arises, if the normal process of getting food is at bottom in the majority of cases a reaction to a chemical stimulus, what is the nature of the chemical substance causing it? Can the same response be induced by the use of different inorganic and organic chemicals? Is there any relation between chemical composition and the intensity or form of the reaction? To answer these and a number of other questions arising out of them recourse must be had to experiments in which the nature and concentration of the chemicals affecting the organisms may be controlled. All the experiments of this kind I will group together under the heading—

b. Reactions to Chemical Stimuli—Chemotaxis.

1. Reactions to Localised Chemical Stimuli.—This particular phase of the general subject of the effects of chemicals may be considered first, since it is most closely related to what has preceded on the food reactions. The plan of the experiments was to try the effect of a series of substances when applied to restricted areas of the body. A sufficiently large number of chemicals were used to include representatives from each of the main groups of substances which have been found to have marked effects on organisms.

a. Methods.—The method which was found to give the most satisfactory results in the application of localised chemical stimuli was the use of a capillary tube filled with the solution whose effects it was desired to test. The form of the tube used is shown in Fig. 32. The tubes were 10 to 15 cm. long, and were made from glass tubing of about 2.5 mm. internal diameter. Each end was drawn to capillary fineness, and then broken off so as to give an opening of the desired size. The opening at the upper end was made

slightly larger than that at the lower, which was used in giving the stimulus. The tube was filled with solution by suction. The rate of diffusion can be regulated by changing the sizes of the openings, and can be determined for each tube from the rate at which the fluid sinks at the upper end of the tube. Considerable experimenting is necessary in order to get the best rate of diffusion for work on planarians. Since the animal is moving rather rapidly while the stimulus is being applied it is necessary to have reasonably rapid diffusion or the worm will not react at all, or not for so long a time after the stimulation has begun that one cannot be certain of the results. It is easily possible to get the capillary so fine that no results can be obtained. On the other hand, when it is too large the solution affects too large a portion of the body at one time, and furthermore, as will be shown later, may cause a rheotactic reaction of the organism. This, of course, introduces a possible source of



FIG. 32.—Glass tube used in giving localised chemical stimuli.

serious error. It can be avoided by frequent and proper control experiments.

It will be well to describe in advance the conduct of a typical experiment and the precautions taken, so that it may not be necessary to repeat these details in the account of each experiment. Six to ten normal active planarians were taken from the aquarium dish and put in a Petri dish of about 10 cm. diameter, in freshly drawn, filtered tap water. Enough water was put in the dish to give a depth of about 1 cm. Two or three of the capillary tubes with different sized openings were filled with the test solution. These tubes were all tested before a final experimental series was begun, and usually only one which had been found to allow diffusion at the satisfactory rate was used. In some cases, however, varying degrees of sensitiveness among the different specimens made it necessary to use for some in-

dividuals capillaries of faster or slower rates than what may be called the standard. After preliminary experiments to determine the relative sensitiveness of the different parts of the body to chemicals, attention was devoted almost entirely to stimulation of the head region, and consequently in the experiments which will be reported first the stimulus was applied only to the head, unless otherwise stated. The method of applying the stimulus was to place the point of the capillary tube a short distance (about 2 mm.) from the place on the body to be stimulated. The animal was stimulated as it was gliding along in the normal way, and hence it was necessary to move the capillary tube at the same rate the animal moved in order to keep it opposite the same point in case the reaction was not given at the instant the capillary was put into place, which, of course, almost never happens. With a little practice one can move the tube along as the worm glides so as to keep the relative position of the two almost identically the same. Just as soon as a reaction had been obtained with a given specimen the capillary tube was removed from the water, so as to permit as little as possible of the chemical to get into the water surrounding the organism. After a series with any substance, the worms were transferred at once to a dish of fresh water before beginning another series. Further, in any long series, when for any reason it might be supposed that the water was becoming contaminated with the chemical to an extent sufficient to affect the results, the worms were transferred to another dish of fresh water. All through the course of an experiment frequent control tests were made by trying the effect on the worms of the water surrounding them when diffusing out from the same tube used previously for the chemical. After each experiment the tubes were thoroughly rinsed by drawing distilled water back and forth through them many times. The tubes were also frequently discarded and new ones substituted.

The following substances were used in the experiments :

Mineral acids	.	{ Nitric Hydrochloric Sulphuric
Organic acids	.	{ Oxalic Citric Formic
Alkalies	.	{ Sodium hydrate Sodium carbonate
Salts of heavy metals		{ Copper sulphate Zinc sulphate
Other salts	.	{ Sodium chloride Sodium bromide Potassium chloride Magnesium chloride
Cane-sugar.		
Distilled water.		

Since distilled water was found to have a decided effect in producing a reaction, the solutions were prepared in both distilled water and in filtered tap water. In case of any doubt, as with very dilute solutions, the effects of the solutions prepared in each sort of water were tested and compared.

Since only qualitative results were desired, and for the practical reason of greater convenience, percentage rather than molecular solutions were used.

β. Results.—The results are, in a way, so remarkable that they will be presented in some detail.

Mineral Acids.

Nitric (sp. gr. 1.42), $\frac{1}{5}$ per cent.—This solution causes strong negative reaction. If applied to the head region the animal turns away from the side stimulated immediately, and strongly. If the stimulus is long continued the animal writhes and twists about violently.

Stimulation of the posterior region causes the part where

the solution strikes to contract very violently, and the whole animal to start crawling ahead rapidly. This concentration is very injurious, and if its action is continued, quickly kills the individual. It will be noted that its effects are the same essentially as those of strong mechanical stimuli applied to the same parts of the body.

$\frac{1}{10}$ per cent. and $\frac{1}{20}$ per cent.—Results the same as in $\frac{1}{5}$ per cent. The animal is not as quickly and extensively injured by these solutions as by the former. It is to be noted that with these comparatively strong solutions the reaction time after stimulation of the posterior end of the body is so slow that this part of the body is permanently injured or destroyed before the animal gets away.

$\frac{1}{40}$ per cent.—In some cases a well-marked positive reaction was caused by stimulation of the head region with this solution. The head would turn towards the mouth of the pipette in the characteristic fashion of the food reaction, or the reaction to weak mechanical stimuli. In other individuals the reaction given was weakly negative, while still other specimens were indifferent. In cases where there was an indifferent reaction there was a local contraction of the side of the head stimulated.

$\frac{1}{80}$ per cent.—Clearly marked positive reaction in large majority of cases after the stimulus has acted for some time. This solution never caused the negative reaction. Some individuals were, in a few cases, indifferent to this solution. This solution is too weak to start a resting specimen into movement.

$\frac{1}{160}$ per cent. and weaker.—Indifferent reactions or weak positive.

This acid appears to be a very strong stimulus for the negative reaction in concentrations down to $\frac{1}{40}$ per cent., while below that it is a rather ineffective stimulus, and the reaction when induced is positive.

Hydrochloric, $\frac{1}{10}$ per cent.—Strong negative reaction. There is noticeable in some cases a tendency for some individuals to turn very slightly towards the source of

stimulation before giving the strong negative reaction. Stimulation of the anterior end of a decapitated specimen caused a slow negative reaction with long reaction time. This solution causes the change from the glide to the crawl when applied to the posterior end of a normal worm.

$\frac{1}{20}$ per cent.—Negative reaction; rather weaker than with preceding solution. With this solution one specimen would turn towards the source of the stimulus until the head came into the strong acid near the mouth of the pipette, and then give the sharp negative reaction.

$\frac{1}{40}$ per cent.—Specimen A gave positive reaction in every case; specimen B in about 50 per cent. of all cases, while in the remainder of trials gave weak negative. Other specimens negative reaction.

$\frac{1}{80}$ per cent.—Specimen A as in preceding case. Specimen B gave positive reaction in about 90 per cent. of all trials. Other specimens weakly negative reactions.

$\frac{1}{160}$ per cent.—All specimens give well-marked positive reaction. They glide up to the end of the capillary and "grip" it with the anterior end as in the food reaction. After holding on for a moment they let go and give a sharp negative reaction, indicating that the stimulus is still too strong when continued. This behaviour will indicate the machine-like character of the positive reaction.

$\frac{1}{320}$ per cent.—In the majority of cases indifferent reaction. Remainder positive.

To give an idea of the dependence of the reactions to chemicals on the physiological condition of the organism, the following series of experiments with HCl in solutions of $\frac{1}{160}$ per cent. and weaker concentrations may be described. It is to be understood that these experiments were carried out on different animals from those just given.

$\frac{1}{160}$ per cent.—No sharp positive reaction. Specimens will give a weak negative reaction if the opening of the capillary is held very near the head. In most cases reactions are indifferent.

$\frac{1}{320}$ per cent.—One specimen gives positive reaction and

goes through whole food reaction on the end of tube. The remainder still give weak negative reactions.

$\frac{1}{640}$ per cent.—Reactions essentially the same as in $\frac{1}{320}$ per cent.

At this point this series was discontinued. It shows that any absolute concentration for a chemical solution which will cause all planarians to give the positive reaction cannot be assigned. How a given individual will react to a given concentration of chemical depends almost, if not quite as much, on the individual as it does upon the solution.

Sulphuric, $\frac{1}{10}$ per cent. and $\frac{1}{20}$ per cent.—Caused immediate and violent reaction. Decapitated worm reacts like normal. This is evidently a very strong stimulus.

$\frac{1}{40}$ per cent.—Caused strong negative reaction in majority of cases. One specimen reacted as follows:—the capillary tube being held some distance away from the head, it first gave a well-marked positive reaction. On coming into the stronger solution near the mouth of the tube it began strong convulsive contractions (evidently on account of too strong stimulation). It remained, however, at the same spot, and after a few minutes extruded the pharynx and swept it about over the bottom. The specimen remained this way for some time. The tube was, of course, removed immediately after the first positive reaction was given. A decapitated specimen in one case gave a very distinct positive reaction to this solution, the tube being held some distance away from the specimen.

$\frac{1}{80}$ per cent.—Negative reaction. Decapitated specimen gave positive reaction once. This solution, applied to the posterior end of the body, induces the crawling movement.

$\frac{1}{160}$ per cent.—Negative reaction. Isolated pharynx contracts into a ball when stimulated with this solution.

$\frac{1}{320}$ per cent.—Positive reaction in one case. Remainder negative. Same result with pharynx as in $\frac{1}{160}$ per cent.

$\frac{1}{640}$ per cent., $\frac{1}{1280}$ per cent., and $\frac{1}{2560}$ per cent.—With these solutions the reactions were for the most part negative.

In a few cases positive responses were produced, but not regularly.

$\frac{1}{5120}$ per cent. — Positive reaction in all cases. The whole food response was produced in case the end of the tube was left in position. The worms "gripped" it, glided up on to it, and extruded the pharynx, in many cases running the latter up into the lumen of the tube. Anterior piece, resulting from cutting animal in two transversely, acts like whole worm (positive reaction), but less strongly. Decapitated worm gave no response. In order to make sure that in this case it was the extremely diluted acid which was producing the result, numerous controls with distilled water and culture water and fresh tap water were tried on the same specimens, in alternation with trials with the acid. With tap water and culture water the specimens were indifferent; but with the acid solution ($\frac{1}{5120}$ per cent.) mixed in either tap water or distilled water they gave a well-marked positive reaction. This showed clearly that the results were due to the acid.

Summary.—With the three mineral acids tested it was found that to concentrations above a certain point the specimens always gave the negative reaction, while to concentrations below this point the positive reaction was given. The absolute value of this "critical point" varies widely with different individuals. The behaviour is essentially the same as that in response to mechanical stimulation, viz. to strong stimuli the negative reaction is given, to weak the positive.

Organic Acids.

Oxalic, $\frac{1}{5}$ per cent. and $\frac{1}{10}$ per cent.—Sharp negative reaction. This solution affords a very strong stimulus and quickly kills the specimen. The negative reaction is very violent when once induced, but several specimens were killed before they turned away. There was noticeable a slight tendency to turn towards the stimulus the instant it was perceived, and before this could be replaced by

the negative reaction the specimens were nearly or quite killed.

$\frac{1}{20}$ per cent.—Convulsive negative reaction in the great majority of cases. In one case stimulation was followed by sharp positive reaction, succeeded by extrusion of the pharynx.

$\frac{1}{40}$ per cent. and $\frac{1}{80}$ per cent.—A few specimens on some trials give positive reaction, and then go into convulsive twisting movements as they get into stronger solution. Remainder negative.

$\frac{1}{160}$ per cent. and $\frac{1}{320}$ per cent.—Positive and weak negative reactions about equally divided.

$\frac{1}{640}$ per cent. and $\frac{1}{1280}$ per cent.—Positive reactions becoming proportionately more numerous. Negative reactions are very weak when given in response to these solutions. In the cases where there is a positive reaction the full response is not given; the specimens go up to the mouth of the tube, but do not grip it nor extrude the pharynx.

$\frac{1}{2560}$ per cent.—With this solution all but one specimen give the positive reaction. Specimens will follow the end of the pipette about the dish if it is moved slowly. This is done by a series of positive reactions. Specimens will give the complete food reaction on the end of the tube.

Citric, 2 per cent.—Strong negative reactions.

1 per cent.—Less marked negative reactions. Tendency to positive in some cases.

$\frac{4}{10}$ per cent.—Positive reactions in nearly all cases. Remainder indifferent.

$\frac{2}{10}$ per cent.—Indifferent.

Citric acid in weak solutions seems to be a very ineffective sort of stimulus, not causing pronounced reactions of any kind.

Formic, $\frac{1}{5}$ per cent. and $\frac{1}{10}$ per cent.—Prompt and decided negative reaction. Causes a resting worm to give a weak negative reaction of the anterior end, but does not start the whole animal into movement, provided the tube is withdrawn after the first reaction is obtained.

$\frac{1}{20}$ per cent.—Negative reaction, but decidedly less pronounced than with preceding concentrations. Does not cause any movement whatever in resting specimen.

$\frac{1}{40}$ per cent.—Negative reaction, less strong than in previous cases. In some cases positive reaction. Noticeable tendency to give slight positive reaction just before the definite negative response.

$\frac{1}{80}$ per cent.—Well-marked positive response.

Summary.—The same conclusions are to be drawn from the experiments on organic acids as from those on mineral acids, viz. that to strong concentrations of a given substance the negative reaction is given, while weak concentrations cause a positive response. Oxalic acid is rather peculiar in that it appears to furnish in all concentrations a stimulus of the proper quality to induce the positive response, but is at the same time excessively harmful in any above the weakest solutions.

Alkalies.

Sodium Hydrate, $\frac{1}{5}$ per cent., $\frac{1}{10}$ per cent., and $\frac{1}{20}$ per cent. — Immediate strong negative response. Specimens turn away very sharply. In $\frac{1}{20}$ per cent. the reaction is slightly weaker than in the other two.

$\frac{1}{40}$ per cent.—Negative reaction. Stimulus applied to posterior end of body is sufficiently strong to cause crawling movement.

$\frac{1}{80}$ per cent.—Weaker negative reaction. Sufficiently strong to start resting animal into movement.

$\frac{1}{160}$ per cent.—Weak negative reaction. Ineffective on resting worm and on posterior end of body of moving specimen.

$\frac{1}{320}$ per cent.—Very weak negative response. In one specimen sharp positive reaction; performs whole food reaction on the end of the tube.

$\frac{1}{640}$ per cent.—Positive reactions from all specimens. The complete food reaction is given.

To solutions below this concentration the organisms are either indifferent or, in a few cases, weakly positive.

Sodium Carbonate, $\frac{1}{5}$ per cent.—Rather weak negative reaction.

$\frac{1}{10}$ per cent.—Majority of all reactions positive. Remainder weakly negative.

$\frac{1}{20}$ per cent.—Well-marked positive reaction in all cases. The specimens can be led around the dish by moving the tube slowly.

Below this concentration the reactions were either indifferent or weakly positive.

Summary.—A caustic alkali (NaOH) and a salt of strong alkaline reaction (Na_2CO_3) produce essentially the same results as the acids. In strong solutions they cause negative reactions; in weak, positive.

Salts of Heavy Metals.

Copper Sulphate, $\frac{1}{10}$ per cent.—At the very first trials the animals all turned sharply and immediately towards the stimulus (positive reaction), but the solution was so strong as to throw the animal into convulsions, when the head came very near the mouth of the tube. Subsequent trials produced the negative response.

$\frac{1}{20}$ and $\frac{1}{40}$ per cent.—All specimens give positive reaction. The head is brought up to the tube, and the worm glides up over the latter.

With all concentrations of CuSO_4 there is a very well-marked local contraction of that part of the body which is stimulated.

In another series of experiments with this same substance, the strongest concentration which would produce the positive reaction in all cases was $\frac{1}{640}$ per cent.

Zinc Sulphate, $\frac{1}{5}$ and $\frac{1}{10}$ per cent.—Sharp and immediate negative reaction.

$\frac{1}{20}$ per cent.—Negative reaction, but less pronounced than in former cases.

$\frac{1}{40}$ and $\frac{1}{80}$ per cent.—Specimens stop when stimulated,

and wave the anterior end about in the water, first away from and then towards the source of stimulation. As the head comes nearer to the end of the tube, where the solution is strongest, it is more strongly stimulated, and gives a definite negative reaction. As it gets out into the weaker zone again it is stimulated to a positive reaction once more. If the tube is now removed the specimen will, in some cases, after a short time turn sharply towards the place where it was, and move in that direction. In other cases the negative reaction finally predominates. It not infrequently happens that in the earlier part of this reaction the anterior end only moves very slightly towards, or very slightly away from the stimulus, so that the body seems, at first sight, to be fixed in one position. The planarian, in this strenuous reaction, probably comes as near to the hypothesized behaviour of the famous "Buridan's ass" as anything is ever likely to in actual practice.

$\frac{1}{160}$ per cent.—One specimen gave clearly marked positive reaction in every case. Others as in the preceding solutions ($\frac{1}{40}$ per cent. and $\frac{1}{80}$ per cent.).

$\frac{1}{320}$ per cent.—Well-marked positive reaction. Specimens give complete typical food reaction.

In one case, with a small worm, I was able to produce crawling in a backward direction by continuous stimulation of the anterior end in the middle line of the body with $\frac{1}{80}$ per cent. ZnSO_4 .

Summary.—The results from solutions of salts of two heavy metals are in accord with those obtained with other chemicals.

Other Salts.

Sodium Chloride, $\frac{1}{5}$ per cent. and $\frac{1}{10}$ per cent.—Negative reaction; distinct, but not as strongly marked as the negative reaction to strong acids.

$\frac{1}{20}$ per cent.—Weak negative reactions and weak positive reactions in about equal numbers. Many of the trials produce no response whatever.

$\frac{1}{40}$ per cent.—Weak positive reactions in nearly every case. No negative reactions. The typical, complete food reflex I have not been able to induce with sodium chloride.

Concentrations below this do not produce any definite reaction.

In general, NaCl is a very ineffective stimulus to planarians, either to the positive or the negative reaction. Distilled water is a considerably stronger stimulus to the positive reaction.

Sodium Bromide, 2 per cent.—Weak but distinct negative reaction in all cases.

$\frac{2}{5}$ per cent.—Well-marked positive reaction in all cases. Complete normal food reaction is produced.

Potassium Chloride, 2 per cent.—The animals usually react in a peculiar way to this and stronger solutions of KCl. When stimulated they stop, turn the anterior end either slightly towards or slightly away from the source of stimulation, and then stay in the same place and squirm and twist the body. In some cases there is a well-marked negative reaction.

$\frac{1}{5}$ per cent.—Some specimens give negative reactions in the first few trials; afterwards give definite positive responses, as do other specimens in all cases. In one case the specimen gave marked positive reaction, and after the head was turned towards the stimulus, remained quiet in the same position as long as the chemical acted.

$\frac{1}{10}$ per cent.—All specimens give positive reaction or are indifferent. The whole food reaction took place on the end of the tube. In this experiment it could be clearly demonstrated that the pharynx is positively chemotactic to this substance. It is probably positively chemotactic to all substances which induce the preceding portions of the feeding reaction. If, after the pharynx had been extruded, the tube was turned about so that the ventral surface of the animal could be seen, and the posterior part of the body was moved with a needle, so as to change the position of the pharynx with reference to the mouth of the tube, it could be

seen that this organ bent directly towards the mouth of the capillary. The pharynx oriented itself with reference to the issuing chemical.

The cases in which specimens were "indifferent" to this solution (i. e. did not give either the positive or negative reaction) were evidently not due to the fact that the animal was not stimulated, but, on the contrary, that it was stimulated about equally to negative and positive responses. This was indicated by their restless behaviour when "indifferent." While the animal as a whole moves in a straight line, the head constantly moves slightly towards and away from the stimulus. Evidently the solution is not quite strong enough to induce a definite negative reaction, nor quite weak enough to cause a clear positive response.

$\frac{1}{20}$ per cent., $\frac{1}{40}$ per cent., and $\frac{1}{80}$ per cent.—Distinct positive reaction in all cases.

$\frac{1}{160}$ per cent.—Positive reactions in some cases, mainly indifferent. The "indifference" is now due to lack of stimulation.

Below $\frac{1}{160}$ per cent. I have been unable to get definite responses of any sort with KCl.

Magnesium Chloride, $\frac{1}{5}$ per cent.—Usually sharp negative reaction. In some cases a slight turn towards the stimulus preceded the negative response, and in some few other trials the animal was indifferent.

$\frac{1}{10}$ per cent.—Weaker negative reaction. In one case clear positive reaction. No local contraction of the region stimulated is caused by this chemical.

$\frac{1}{20}$ per cent.—Positive reaction in all cases. Complete food reaction could be induced.

$\frac{1}{40}$ per cent.—Weak positive reaction or indifferent.

Summary.—To the salts NaCl, NaBr, KCl, and MgCl_2 the planarians react as to other chemicals, by giving the negative response to strong concentrations and the positive to weak.

Cane-sugar.—Sugar solutions, in all concentrations above $\frac{1}{10}$ per cent., so far as I have been able to discover,

cause well-marked positive reactions in all cases. This is the only chemical which I have found that causes only one of the reactions.

Distilled Water.—To distilled water applied by the capillary method the organisms give a well-marked positive reaction in all cases. That the reactions to very dilute solutions of chemicals were not due to the distilled water in cases where this was used as the solvent, rather than to the chemical itself, was proven in the following way :—Parallel experiments were performed, using tap water as a solvent, and in every case the same reaction was given to the tap-water solution as to that in distilled water. At the same time the specimens would not react to clear tap water applied in the same way by the same tube.

2. General Summary.—Putting all the results on the effects of localised chemical stimuli together, we are forced to the somewhat remarkable conclusion that practically all substances are both “attractive” and “repellent” to planarians. Evidently, then, the chemical composition of a substance is not of the first importance in determining how the individuals shall react to it; but, on the contrary, its concentration is the important matter. To weak solutions of any chemical the animals give positive responses, while to strong solutions they give negative.

Between the behaviour towards chemical stimuli and towards mechanical stimuli there is a very close parallelism, or, perhaps better, identity, which is evidently something of fundamental importance. In order to bring this out more clearly it may be well to arrange in tabular form the results of the study of the reactions to these two stimuli.

	Mechanical Stimuli.		Chemical Stimuli.	
	Strong.	Weak.	Strong.	Weak.
Unilateral stimulation of head region	Negative reaction	Positive reaction	Negative reaction	Positive reaction.
Stimulation of head region on median line	Either a very strong negative reaction, or crawling backwards	Positive reaction	Strong negative reaction, or crawling backwards	Positive reaction.
Stimulation of middle region of body	Essentially the same as for stimulation of the head		The same as for stimulation of the head, except that the sensitivity is much less, and diminishes more rapidly posteriorly than in case of mechanical stimuli.	
Stimulation of posterior region of body	Crawling ahead	Local contraction	Crawling ahead	No effect, or slight local contraction.

From this close parallelism we must conclude, I think, that in the behaviour of planarians the qualitative character of a stimulus is of little importance in comparison with its quantitative relations. Or, to express it differently, to all stimuli which are of low intensity the flat-worm gives the positive reaction, while to stimuli which are of high intensity it gives a negative response. This sort of behaviour will at once be seen to be, in the long run, purposive, and is, further, of a kind which might very well have been developed by the action of natural selection. In the long run the planarian's reactions will take it away from injurious substances and into favourable surroundings.

These results on chemicals are interesting in connection with the work so much done in recent times on the specific

effects of ions and the conclusions based on very fine quantitative results with chemicals. Two such series of experiments as those quoted above from HCl and CuSO_4 indicate what would be the worth of the assignment of an absolute value for the concentration of either of these two substances which would produce the positive reaction in planarians. Such instances might be multiplied, and they serve to bring out the fact, apparently so frequently lost sight of, that what an organism will do when stimulated is quite as much a function of the physiological condition of the organism itself at the time as it is of the stimulus.

A comparison of these results with those of Yerkes (: 02) on the reactions of *Gonionemus* is of much interest. This author finds that though there is a well-marked and characteristic food reaction, which is given in response to food substances, whether in solid or liquid form, yet this reaction cannot be induced by other chemicals. It is stated that a number of chemicals were tried in all concentrations for the special purpose of determining whether the food reaction might not depend upon intensity rather than quality of stimulus. This was not found to be the case. We must, then, conclude that *Gonionemus* is a stage farther along in its psychic development than is the flat-worm, for the medusa reacts with reference to the quality as well as to the intensity and location of the stimulus, while with the flat-worm the intensity and location of the stimulus are by far the most important factors. It is necessary in the case of the flat-worm, to be sure, that there be mechanical and chemical stimuli acting together in order to produce the complex of reflexes forming the complete food reaction, thus indicating some relation to quality of stimulus. But for the production of what is, in one sense, the most important phase of the reaction, the turning towards the source of stimulation, the quality of the stimulus is not significant.

With an understanding of the method of reaction to localised chemical stimuli, a number of interesting special problems present themselves. While it will not be possible

to take up all of them in this paper, a few of the specially important ones may be considered.

One such important general question which arises is the problem of orientation to diffusing chemicals. Do planarians orient themselves along radial lines of diffusion and proceed towards the centre of diffusion? It would seem that in the case of such a perfectly bilaterally symmetrical organism as *Planaria*, if anywhere, Loeb's theory of orientation ought to hold good. This theory accounts for orientation by supposing that when an organism is stimulated unilaterally its motor organs are caused to act either more strongly or more weakly, as the case may be, on that side than on the other. This results in bringing the long axis of the body parallel with the lines of action of the stimulus; and then, since symmetrical points on either side of the body must be equally stimulated, the organism moves in a straight line towards or away from the stimulus. Jennings has shown (: 01) that for most stimuli this theory of orientation does not hold in the case of the Infusoria.

From the account of the reactions of planarians to chemical stimuli which has been given, it will be at once seen that there is in this case, to some degree at least, an orienting reaction. With weak chemical stimuli the head turns towards the stimulus in such a way as to point the anterior end very directly towards the source of stimulation. It might be thought that this marked a pure orientation, but it must be remembered that the organisms turn the head just as precisely towards the point from which a weak mechanical stimulus comes. The two reactions are evidently exactly the same thing. However, a single mechanical stimulus can hardly be considered a directive stimulus of the sort which induces an orientation, such as, for example, the electric current. The orientation of unicellular organisms to the constant current is the purest type of an orienting response, however, and the most characteristic thing about it is that the organism, after having the anterior end turned towards one of the poles, keeps the long axis of the

body parallel to the lines of action of the stimulus. This movement of the animal in a constant relation to a constantly acting stimulus is, as I understand it, the fundamental criterion of an orientation according to the theory above mentioned. Now if we find, as has been shown above to be the case, that the organism gives precisely the same reaction to a chemical unilaterally applied as it does to a single weak mechanical stimulus similarly applied, it seems doubtful whether we can consider that there is such an orientation in the case of the chemical, even though the head is directed very precisely towards it. On the contrary, it seems apparent that we are dealing here with a well co-ordinated motor reflex only—such as, for example, the reflex of a frog's hind leg, which brings its foot very exactly to the point stimulated on the side of the body.

A crucial test of this point may be obtained by submitting the animals to the action of some chemical to which they are known to give the positive reaction when it is applied locally, only arranging the experiment so that it is diffusing over a large area. Under these conditions, if the organism shows positive orientation, it ought to move along the lines of diffusion straight up to the source of diffusion. To test this matter I constructed a trough of the form shown in Fig. 33, I. On a plate of glass A was fastened the trough B, which was cut from a block of paraffin. The internal dimensions of this trough were 50 mm. \times 50 mm. \times 5 mm. Only the sides were of paraffin, the glass plate serving as the bottom. A hollow was cut in one end of the trough, and a glass tube D, about 4 cm. long, was fastened into it in an upright position. Then from the point *x* on the inside of the trough a fine needle was thrust through the paraffin till it came out into the hollow previously cut in the wall. A sectional view of this part of the device is shown in Fig. 33, II. When it was desired to use the apparatus the trough was filled with filtered tap water and a number of planarians placed in it. Then into the tube D was introduced a certain amount of the solution whose effects were to be tested. By varying the amount of

the solution introduced, the rate of its diffusion through *x* into the water could be very nicely controlled. This matter was thoroughly tested, and the apparatus in a sense calibrated by the use of coloured solutions before the actual experiments were begun.

A considerable number of experiments were tried with this diffusion trough, with the following results:—In no case was there any observable orientation of the organisms. A typical experiment will illustrate what actually took place. A $\frac{1}{20}$ per cent. solution of Na_2CO_3 , which by the capillary method always produces a sharp positive reaction, was put

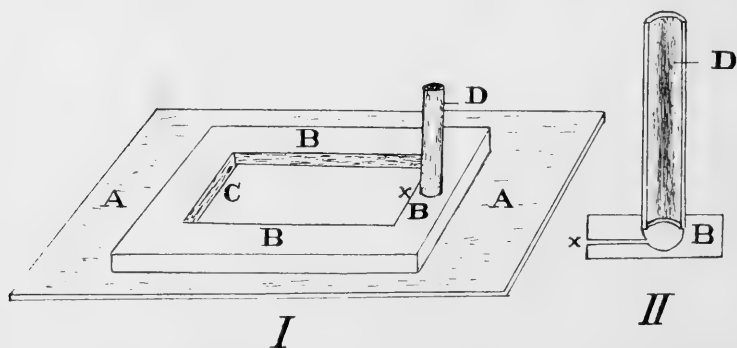


FIG. 33.—I. Diffusion trough used for testing the reactions of planarians to diffusing chemicals. A, A. Glass base plate. B, B. Paraffin trough. *x*. Point of opening of diffusion tube. C. Cavity of trough in which the specimens are placed. D. Tube in which the solution to be tested is placed. II. Enlarged sectional view of the end of the trough bearing the diffusion tube. Lettering as in I.

into the tube D in sufficient quantity to give a diffusion of moderate rate. After it had been diffusing for some time (by test with coloured solutions long enough to reach the middle of the trough) specimens were introduced at the end C. They started gliding about in random directions at once. Some passed diagonally up to the end D; others remained nearer the end C; while still others went up on the paraffin sides to the end D. None went straight towards *x* after they had come into the region where the chemical had diffused. No reaction of any sort was given in the course of the passage

towards the end D in the majority of cases. In some few instances an individual would give a weak positive reaction (i. e. turn slightly towards x) at some point in its course, but this was so small in amount that it did not in most cases turn the animal directly towards x . Further, the direction of movement was frequently changed considerably, and turned away from x after this weak positive response. In other words, the animals moved about in the trough practically at random, giving only slight reactions in a few cases while in the area of diffusion. Many of the individuals, after reaching end D of the trough, turned around and went back to the other end again, just as they would have done provided no chemical had been present. Other specimens would glide across the trough on the paraffin of the end D. Only these specimens showed any definite response to the chemical. When they came within the length of their own bodies from the opening x they gave a well-marked positive reaction and went to x . Having arrived there, they explored and "gripped" the edge of the hole with the head, and then extruded the pharynx. The pharynx was usually stretched up into the diffusion opening, and the worm proceeded to feed for a time on Na_2CO_3 .

These experiments were repeated many times with a variety of chemicals of various concentrations, and diffusing at various rates. It was very certain in all cases that there was no definite orientation along lines of diffusing ions. When the organism by chance came near the diffusion opening x , it would give a positive reaction if the solution was of the proper concentration, and then proceed to give the complete food reaction over the hole, but there was no continued orientation.

There was a similar absence of a negative orienting response when strong solutions of acids were used. In this case the animals stayed at end C of the trough, but this was because when, in the course of their random movements, they struck the diffusing chemical where it was of sufficient concentration, they gave the usual negative reaction, turning the anterior ends about 30° away, and starting off on the courses

so defined. If they came in contact with the strong solution again they repeated the reaction. In no case did they turn squarely around with their heads directly away from *x* and the long axis parallel to the lines of diffusion.

It would be unprofitable to further multiply accounts of these experiments, since all led to the same result. No definite orientation occurred, but only the positive and negative motor reflexes coupled with random movements. Whether, as some maintain, we have in these positive and negative reactions the "Dinge an sich" of orientations is a question for the metaphysician rather than the physiologist to decide. The objective reality of the matter is that in the behaviour of planarians towards chemicals there is no orientation in the lines of diffusing ions, i. e. no phenomenon like the orientation of *Paramecium* to the electric current.

Another problem of importance in connection with the reactions of the organisms to chemicals has to do with the formation of collections of individuals. Are collections formed in certain chemicals, as is the case with certain of the Infusoria as described by Jennings? As this author has set forth, *Paramecia* will form dense aggregations in drops of various chemicals, particularly weak acids, introduced into the culture water. The method by which this is done is as follows:—Individuals swimming about at random strike the drop of acid by chance and pass into it without giving any reaction; when, however, they come to the opposite side of the drop, and start to pass from it to the water again, they are stimulated and give their characteristic motor reaction (jerk back and turn towards the aboral side). This reaction turns them back into the drop, which forms, as it were, a trap for all that enter it. In a short time a dense aggregation is formed. This is almost the only method of active reaction, known aside from orientation, which will produce collections of organisms in chemicals. Its essential feature is not the getting of the organisms into the chemical, this being purely a matter of chance, but the holding of them in the chemical after they have entered it, by what

amounts to a negative reaction to the surrounding water. The question, then, is, can we get any such formation of collections by the retention of those specimens which have entered an area by chance in the case of *Planaria*?

This problem was attacked in a number of different ways, but the clearest results could be obtained by the "two-drop" method of Massart. Two drops of fluid of equal size are placed near each other on a slide, and a narrow connecting band is made between the two by drawing some of the fluid across with a needle. One of them was usually of culture water, while the other was of the solution to be tested. Now evidently, if the animals form collections by the "motor reflex" method, they ought to pass into the drop of solution without any reaction, but when they attempt to pass back into the water drop they should be stimulated to a negative reaction and thus turned back.

An experiment with a solution to which the animal gives a sharp positive reaction may first be reported. One of the drops was tap water, and the other was 1 per cent. sugar solution, to which the specimens gave a strong positive reaction. Several small planarians were put into the water drop. They glided rapidly about this drop, and soon one came up to the bridge connecting the water with the sugar. It was headed straight for the sugar drop, and passed over into it without any reaction whatever. Up to this point the behaviour is like that of the Infusoria towards the acid drop. This specimen circled about in the sugar drop, and after a time became directed towards the connection between the sugar and water, and passed back into the water drop without giving the faintest trace of a reaction of any sort. All the specimens passed back and forth between the two drops without giving any reaction, except in some cases a weak positive one. The conditions under which a positive reaction is given are that a specimen should come more or less transversely across one end of the connecting bridge, as shown in Fig. 34. It then usually gives a weak positive reaction and turns slightly towards the other drop. It may do this on

passing either from the water to the sugar or vice versâ. When in sugar solution it gives a positive reaction to tap water, whether applied by the capillary tube method or as just described. It is evident, from this experiment, that collections are not formed by planarians in the same way that they are by Infusoria. The animals are not negative to the surrounding water after they have been in the solution. To test and verify this conclusion the experiment was repeated with solutions of different substances. It was found that in case of all substances in concentrations to which the animals gave a positive response when stimulated by the capillary method, the specimens would pass back and forth from water to solution and vice versâ, indifferently. If solutions were used in concentrations to which a negative reaction was given

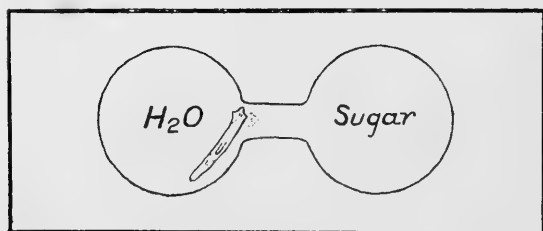


FIG. 34.—Diagram showing the arrangement of "two-drop" experiment with chemicals.

when stimulation was by the capillary method, the specimens merely stayed in the water drop. When they came to the boundary line of the strong solution they gave the negative reaction, and hence stayed in the water. This immediately raises the question, why would there not be a permanent collection of the planarians formed in a drop of a substance to which they give the positive reaction, provided they were first put in a drop of some substance to which they were strongly negative? There is evidently no theoretical reason why this should not take place, but there is an important practical one. This is that any solution which would cause a negative reaction, under these circumstances, will, so far as I have found, also seriously modify the animals' movements, if

they are immersed in it. They will simply squirm about and make no progressive movements, and hence not get into the drop of substance to which they are positive. But it is quite possible that by making a long enough series of experiments on this point, one might get a solution just strong enough to cause a negative reaction, and in which the organisms would still move well. We would then get a collection in the positive drop. The important thing, however, is that to the water in which they live the animals do not, under any circumstances, give a negative reaction, and hence under normal conditions no collections can be found by a "motor reflex" method.

It may be well, before leaving this subject, to point out the fundamental physiological difference between the Infusoria and the planarians, on which the difference in the behaviour towards chemicals is based. It is that in the case of the Infusoria there is but one form of reaction (the "motor reflex" turn towards a structurally defined side) regardless of whether the stimulus is strong or weak, while in the case of the planarian there is a qualitatively different reaction to strong stimuli from that which is given to weak. When the infusorian passes into the drop of acid it is apparently not stimulated at all (for what reason we do not know). When it attempts to pass from acid to water it is given a stimulus which must be in the nature of things a rather weak one, yet it responds with the only reaction it has, and is, as a consequence, kept in the acid. With the planarian any slight change in environmental conditions gives a weak stimulus, and the specimen turns towards the source of stimulation. This serves, together with random movements, to get it into the drop of solution; but when it strikes again the water, which again must furnish a weak stimulus, it gives the same positive reaction and passes out into the water. The ability to differentiate in the reactions between the strong and weak stimuli gives the organism a far greater range in its activities.

Another problem which is of interest in connection with

food and chemical reactions is the relation of the condition of the organism as regards hunger to its reactions to stimuli. It might be supposed that an individual which had not had food for some time would be more apt to give the positive reaction to a given stimulus than one which had just fed.

To test this point parallel experiments were instituted with specimens allowed to feed till they left the food spontaneously about three hours before the experiments, and specimens which had been kept for three weeks in a dish of clear water. NaBr was used as the stimulating solution, and was applied by the capillary method. The specimens chosen were of the same species, *P. dorotocephala*, and as nearly as possible of the same size. The only difference which could be detected between the fed and the unfed animals in their behaviour towards a $\frac{2}{5}$ per cent. solution of NaBr was that the unfed animals gave the whole food reaction on the end of the capillary tube, while the recently fed specimens only went so far as to give the positive reaction, and touched the end of the tube with the anterior end of the head. They did not "grip" it and pass up on to it, as did the others. In the main point at which I was working, namely, the giving of the definite positive reaction, there was no discoverable difference between the fed and unfed specimens. One set gave the reaction just as promptly and decidedly as did the other. Next a weaker solution, $\frac{1}{10}$ per cent., was tried. With this solution about 50 per cent. of the specimens in ordinary condition give a weak positive reaction, and 50 per cent. are indifferent. This concentration, being about on the border line between that which affords no stimulus at all and that which is a definite stimulus for the positive reaction, ought to bring out any differences which may exist between fed and unfed individuals in the sensitivity to stimuli for the positive reaction. As a matter of fact, no difference in the behaviour of the two sets was to be observed. One gave a well-marked positive reaction in as many cases as did the other. In some instances the reaction time of the fed specimens seemed to be slightly greater than that of the

unfed, but this was neither marked nor of general occurrence. This experiment was afterwards repeated with other specimens, and with sugar as the stimulus, with essentially the same results. I have also repeatedly tried stimulating with various solutions specimens which had just ceased feeding, and in these cases found no certain difference between their behaviour and that of specimens which had not been fed for some time, with regard to the giving of the positive reaction. It would appear, then, that so far as the giving of the positive response to weak stimuli is concerned, the amount of food the animal has previously had is of very little consequence. The failure of fully fed specimens to give the full feeding reaction on the end of the capillary tube indicates that the physiological changes induced by recent feeding affect the performance of the food-taking rather than the food-seeking reflexes.

3. Unlocalised Action of Chemicals.—An extensive

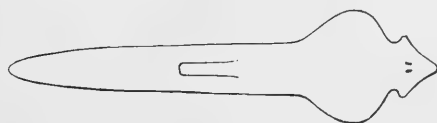


FIG. 35.—Diagram showing the form of crawling movement exhibited by *Planaria* when placed in 10 per cent. NaCl.


series of experiments on the effects of immersing planarians in various solutions was performed, but as the results threw but comparatively little light on the general nature of the behaviour, they will be reported only briefly. Immersion in any strong solution causes marked changes in the movements. The gliding is made very much slower or entirely disappears. In 10 per cent. NaCl a peculiar form of crawling appears. Very pronounced contraction waves pass over the body longitudinally, giving it the appearance shown in Fig. 35. In 2 per cent. CuSO_4 the animals make no progressive movements, but wave the head violently from side to side. In strong solutions of acids the worms squirm violently without making any effective progressive movements. In all these strong solutions the sensitiveness to all stimuli is

greatly diminished. This can best be shown with mechanical stimulation. In strong solutions of NaCl (10 per cent.) the animals make no attempt to right themselves if placed with their dorsal surfaces down. Another peculiar effect of strong solutions of NaCl is to cause the extrusion of the pharynx. This organ is thrust out of the body and extended to a much greater length than is usual. Immersion of the animal in weak solutions that cause the positive reaction—as, for example, 1 per cent. sugar—has no definite effect on the movements, but when in these solutions the animals will give the positive reaction to tap water when the latter is applied by the capillary tube method. Under such circumstances contact with water is a slight environmental change, and acts as a weak stimulus.

III. Thigmotaxis and the Righting Reaction.

a. Thigmotaxis.—If a specimen of *Planaria* is turned over and placed dorsal side down on the bottom, it will immediately right itself. This is done by a very characteristic reaction, and is one of the first things to attract the attention of one studying the behaviour of the organism. Loeb ('94, pp. 251—252) held that the righting reaction in the polyclad *Thysanozoon* was due to the negative and positive thigmotaxis (“stereotropism”) of the dorsal and ventral surfaces respectively. The evidence offered for this view was that when the thigmotactic relations of these two surfaces were reversed, the animal reacted strongly, and that this result could not be due to any effect of gravitation, since the animal assumed all possible relations to gravity, and kept them for considerable periods of time. It seemed to me desirable to get, if possible, some further evidence on this subject, and to work out the mechanism of the righting reaction.

That the dorsal surface of the animal is negatively thigmotactic is certain, and can be shown in other ways than by laying the animal on its dorsal surface. For example, if a piece of cover-glass be gently laid on the dorsal surface of

either a resting or a moving specimen, it will very promptly move out from under it. Further, if crevices are arranged of this form () by supporting cover-glasses at two corners, and letting the two opposite corners rest on the bottom of the dish, specimens will not go into them. The moment the dorsal surface touches the cover-glass above, the worm begins to react violently, changing its direction of movement, and goes out from under the cover.

With the existence of an apparent negative thigmotaxis of the dorsal surface established, however, there still arises the question as to whether this is the sole cause which induces the inverted animal to right itself. The following experiment throws light on this point:—A specimen is placed ventral side up on a dry spatula in the air, and then the spatula is placed just beneath the surface of the water in a tall jar or large test-tube and quickly pulled out from under the worm, so that the latter starts falling through the water in an inverted position. Another way in which the worm may be started falling ventral side up is by holding it on a scalpel point above the water, and then dropping it beneath the surface in the desired position. Before the worm has dropped any great distance it will give the characteristic righting reaction, and turn itself over so as to bring the ventral side down again. This is done in precisely the same way as when the animal is inverted on the bottom (to be described later). After the falling animal has thus righted itself it may again give the same reaction, and thus turn itself over so that the dorsal side is down again. In a few cases I have seen a worm after righting itself the first time keep right side up during the remainder of the fall. The most usual behaviour is for the animal to keep giving the righting reaction all the time that it is falling, although this does not, of course, keep it all the time with the same side uppermost. I have performed a large number of these dropping experiments in which the animals were started in both upright and inverted positions, and in all cases they gave the righting reacting one or more (usually more) times

before reaching the bottom, provided the distance through which the drop was made was greater than 7—10 cm. This result seems to indicate that there is something more concerned in the righting reaction than the negative thigmotaxis of the dorsal surface for the following reasons:—(1) the dorsal surface is not in contact with any solid of this experiment; (2) it is in contact with water only, just as is normally the case when the animal is right side up. It may be objected that the experiment is not conclusive, because, as a result of the falling, there is an increased water-pressure on the dorsal surface, and this may act as a thigmotactic stimulus. This objection is met by two different facts. First, the animal gives the righting response in some cases while falling ventral side down, under which circumstances there can be no increased pressure on the dorsal surface. Second, if a stream of water from a pipette is directly squarely against the dorsal surface of a worm normally gliding about on the bottom the righting reaction is not induced, regardless of the force of the stream. Evidently this stream of water against the dorsal surface produces a pressure on the dorsal surface similar to that when the animal is falling, and if the righting reaction in the falling is due to increase of pressure on the dorsal surface, we might suppose that some indication of it would be produced in this case. As a matter of fact it is not. We must conclude, then, that the righting reaction is due, at least in very large part, to some other cause than the negative thigmotaxis of the dorsal surface. This is indicated also by the fact that when solid bodies are laid on the back of a specimen in its normal position, the reaction which is caused is not the righting action, as would be expected if the latter were due solely to the negative thigmotaxis of the dorsal surface. The righting reaction is clearly not due to gravitation, since the flat-worms move on the surface film with the dorsal surface downward. This leaves, as the only factor to which the reaction can be due, the positive thigmotaxis of the ventral surface. I am convinced that it is to this factor that

the reaction is chiefly due. While the negative thigmotaxis of the dorsal surface plays some part in the reaction, it is, as the experiments described above show, a comparatively unimportant factor. The specific relation of these two factors to the definite righting reaction will be brought out in the next section, in which the form and mechanism of this reaction will be set forth.

b. The Righting Reaction.—The righting reaction is a very characteristic piece of behaviour, and can best be described in a single phrase by saying that when the animal is placed on its back it throws itself into a spiral in such a way that the ventral surface of the head comes into contact with the bottom. This ventral surface then attaches itself to the bottom by means of the mucous secretion, and starts gliding ahead. As it goes forward it unwinds the remainder of the spiral, as each successive posterior part of the ventral



FIG. 36.—Showing the form taken by Planaria in the righting reaction.

surface comes into full contact with the bottom. The form of this spiral just after the ventral surface of the head has come into contact with the bottom is shown in Fig. 36. The spiral is thrown very quickly after the dorsal surface touches the bottom, and usually includes the whole length of the body at once. However, by observing a specimen in which it takes place a little more slowly than usual, it can be seen that the movement is started at the anterior end. Beginning with, for example, the right side of the head, this is turned under, while at the same time the left side is raised. This, of course, brings the ventral surface of the head region down, and at the same time makes a twist in the body, just back of the head. In some cases this is the only twist that is made, while in others another similar twist is thrown in the body farther back. As the anterior end after it is righted glides

ahead, the spiral is unwound by the raised edge of each twist dropping down and attaching to the bottom as soon as it is in a position where this is possible. Thus, of course, when the animal has traversed a distance equal to its own length it will have come entirely into the normal position again. The reaction is really a rotation of the body on its long axis through 180° . The mechanism of the turning is such that only a part of the body rotates at a time,—first the anterior end, then the portion next behind that, and so on, till the whole animal has turned over. This rotation by sections, as it were, causes the spiral form which the animal takes on in the reaction.

The number of turns into which the body is thrown in forming the spiral varies with the length of the individual, and apparently to some extent with its physiological condition. There may be only a half-turn in the whole body, or there may be one complete turn; or, again, one and a half turns; or, finally, as many as two complete turns in the body. One complete or nearly complete turn, as shown in Fig. 36, is the usual form of the reaction. In large individuals more twisting is frequently seen. Evidently all the twisting that is absolutely essential for the righting of the specimen is the half-turn given by the turning of the anterior end ventral side down.

The determination of the direction in which the spiral is thrown, or, in other words, the side of the body towards which the anterior end turns in order to get right side up, was for some time a very puzzling problem. A collection of statistics on the matter showed that the anterior end twisted towards the right and towards the left ¹ in an approximately number of cases. This is precisely the result which would be expected if the matter were due to chance only, but the reaction did not give the appearance of being a chance matter. Finally, the determining factor was found to be the relation of the dorsal surface to the bottom. A cross-section

¹ In the figure (Fig. 36) the worm is represented with the spiral thrown towards the left.

of the body of *Planaria* has the form shown in Fig. 37. It is convex in outline on the dorsal side, and nearly straight on the ventral. As a consequence of this shape of the dorsal surface the animal when placed in an inverted position very seldom lies exactly on the mid-dorsal line, and if it does at first it almost immediately tips over to one side or the other, so that its cross-section has the relation to the bottom shown in Fig. 37, B and C. It is then found that the side of the body which is in contact with the bottom determines in which direction the spiral shall be thrown. If the right side of the dorsal surface is down the right side of the head will turn under towards the left and the left side will be raised up over towards the right, or, in other words, the head as a whole will rotate from right to left, i. e. in a

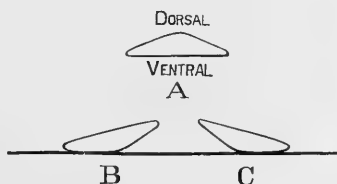


FIG. 37.—Diagrammatic cross-section of *Planaria* to show the contact relations of the dorsal surface of the body to the substrate in the case of a specimen in an inverted position.

counter-clockwise direction. If the left side of the dorsal surface of the body is down at the beginning, the head will rotate from left to right. This relation may be made out easily by direct observation in all cases where the reaction is not too rapid.

The righting reaction is a fairly rapid one. The head is turned over and the spiral thrown in the case of a normal individual almost immediately when the dorsal surface touches the solid. The length of time which it takes a specimen to get completely righted evidently depends on the length of the body, because the longer spiral which must be unwound, the more the time which must be taken. The following figures will bring out this relation between the size of the individual and the time taken in righting. In ten trials with

an active but large specimen (about 12 mm. long) of *P. dorotocephala* the average time taken to regain completely the normal position after being inverted was 8.68 seconds. With a small specimen (5.5 mm. long) the average time taken in righting in ten trials was 5.22 seconds. The time taken in the reaction also depends, of course, on the general physiological condition of the animal. Thus in ten trials with a sluggish specimen, approximately 9 mm. long (thus shorter than the first specimen mentioned), the average time taken in regaining the normal position was 10.90 seconds.

The thigmotactic irritability may be modified or reduced in several ways, and, as a consequence, the righting reaction will disappear entirely or in part. One of these cases has been mentioned above (p. 670) where it was shown that a specimen placed on its back in a 10 per cent. solution of NaCl makes no attempt to right itself. Similarly a specimen put in an inverted position on a dry surface, care being taken that no water surrounds the animal, will not give the righting reaction. In both of these cases the specimens are able to move.

The Mechanism of the Reaction.—It is a very difficult matter to determine exactly the muscular mechanism of this righting reaction, since it is such a complicated movement, and is ordinarily done in its most essential feature—the formation of the spiral—so very quickly. Furthermore, as will appear from the operation experiments to be described, it is almost impossible to devise crucial experiments of a character which will demonstrate what the mechanism is. What I shall do, then, will be to present a tentative explanation of the mechanism of the reaction, together with the evidence for it which I have been able to obtain. I may say that the view to be presented is the result of a long and careful study of the phenomena both in normal and operated worms, and I believe that it is a correct explanation.

The mechanism of the righting reaction is probably as follows:—The half of the body of an inverted specimen which

is in contact with the bottom extends (by the mechanism previously described, pp. 556, 557) in response to the stimulus given by the contact of the dorsal surface of that side of the body with the bottom. At the same time the opposite half of the body, by active muscular contraction, keeps its length the same. Thus any bending of the body away from the side stimulated as in the ordinary negative reaction is prevented, or, in other words, the long axis is kept straight by the opposite side maintaining actively its normal length. Now the necessary mechanical result of keeping one side of a flexible system at a constant length while the other side lengthens must be that the lengthening side will be thrown into a series of waves. In other words, it is mechanically impossible for the lengthening side to keep its whole edge in the same plane. Furthermore, if in such a system it is possible for rotation about a longitudinal axis to occur, the system will be thrown into a spiral of the form which the planarian takes in the righting reaction. Again, as soon as one side of such a system under elongating stress changes its level with reference to the remainder of the system, and thus starts the formation of the spiral, the long axis of the system (i. e. the centre of the spiral) will keep itself straight. Any further force elongating one side will merely throw the spiral into tighter coils without having any tendency to bend its long axis. This fact is of importance in the case of the planarian where the maintenance of the initial straightness of the long axis is done by the opposite side of the body. Of course, a symmetrical spiral cannot be formed unless the two edges are of equal length, but the moment the spiral of the planarian is started all necessity for one side keeping a constant length ceases. It must be kept in mind, however, as has been indicated above, that the force which produces the spiral must act on one side only, and hence the side of the planarian opposite that initiating the movement must be moved passively by the other in the spiral formation after this has once begun. The direction in which the spiral shall turn will evidently not be determined by the mere lengthen-

ing of one side of the body. The determinant of this is evidently a difference of tension on the upper and lower sides, the spiral turning towards the side of greatest tension.¹ This greatest tension is evidently, then, in the normal reaction on the dorsal surface, as we should expect on a priori grounds, since that is the part directly stimulated.

To sum up, the spiral righting reaction of the planarian, as I have worked it out, is due to an elongation of that side of the body whose dorsal surface is in contact with the solid, while the opposite side of the body actively maintains its original length. As the elongation occurs the various parts of the body rotate freely about its long axis, and hence the whole worm takes on the spiral form. The spiral turns towards the dorsal surface in every case (i. e. so as to bring the ventral surface of the head down), as a result of the greater tension of the dorsal musculature on the elongating side.

The reaction is thus seen to be of almost the same character as the ordinary negative reaction to strong mechanical stimuli, in that the primary reaction is an extension of the side stimulated. The difference between the two is that in one case there is a bending of the longitudinal axis of the body, while in the other there is a rotation about this axis. On the view just given of the mechanism of the righting reaction the specific parts played by the positive and negative thigmotaxis of the ventral and dorsal surfaces are evident. The positive thigmotaxis of the ventral surface is the primary cause of the whole reaction, and is evidently the stronger factor of the two, as shown by the experiments of laying solid bodies on the dorsal surface of the animal when in a normal position. It will be recalled that such treatment does not call forth the specific righting reaction. Further evidence of this same thing is found in the fact that speci-

¹ The statements as to the mechanical principles of a spiral have been verified with different sorts of models, including plastic clay, rubber bands, etc. Lack of space will not permit the enumeration of these experiments in detail, but anyone can verify for himself the various statements with very little trouble.

mens will remain in the normal position on the bottom of a dish when there is a layer of plant débris a half-centimetre in thickness above them, and necessarily in contact with the dorsal surface. The negative thigmotaxis of the dorsal surface plays its part in the righting reaction in determining in which direction the turning shall take place.

It has so far been shown that the view of the mechanism of the righting reaction presented is in accord with all the mechanical principles necessary to produce the observed results. The attention may now be turned to an examination of the evidence that this mechanism is the one which actually brings about the reaction. This evidence is obtained from experiments with worms on which operations have been performed. Obviously, if the mechanism described is the one by which the reaction is produced, any operation which destroys or throws out of working order any essential part of the mechanism will cause the typical reaction to disappear, or be greatly modified.

We may first consider the reactions of the pieces resulting from cutting the animal in two transversely in the middle of the body. It is found that each of the pieces resulting from such a cut will perform the righting reaction in the typical manner. The spiral is formed, but there is usually only one half-turn of the body, i. e. just enough to bring the anterior end ventral side down. This then attaches itself to the bottom and starts gliding, unwinding the spiral just as under normal circumstances. There is observable the same relation between the side of the body, which is in contact with the bottom and the direction of the turn as in the normal individual. The only striking difference in the behaviour of the anterior and posterior pieces is that the reaction time of the former is much shorter than that of the latter. The anterior piece rights itself practically as quickly as does the normal animal, while the posterior piece took in one series of experiments 1 minute and 38.1 seconds (average of ten trials) for complete righting. This slower righting reaction is another expression of the generally lowered tonus of such

posterior pieces. By varying the position of the cuts, segments of the body of various lengths may be obtained. All of these, which are about $1\frac{1}{2}$ mm. in length, will usually right themselves by as close an approximation to the typical spiral reaction as is possible under the circumstances. The side of the body which is lowest can be seen to elongate in these very short pieces, and just enough of a twist is found to bring the ventral surface of one corner of the anterior end into contact with the bottom. Of course, no complete spiral can be found in such short pieces. Their reaction time is very slow.

Next, experiments were tried with the pieces resulting from splitting longitudinally anterior halves of worms in the middle line. These pieces had the form shown in Fig. 38. Evidently such pieces have only a half of the mechanism necessary for the performance of the spiral righting reaction,



FIG. 38.—Operation diagram (see text).

according to the view given above, and therefore should not be able to give the typical response. They have one complete side which may elongate, but they have no other side to keep the middle line straight, and so make the elongation effective in forming a spiral. Such pieces, when placed with the dorsal surface down, reacted immediately by bending strongly towards the cut side, i. e., so that the concavity was on the cut side. This was kept up for a time, the animal squirming about violently, but it was finally replaced by another reaction. The ventral longitudinal muscles contracted strongly, and raised the anterior end of the piece well up from the bottom (shown in side view in Fig. 39, *a*). After a strong raising contraction the piece would extend and settle back again. Then after a time the raising was repeated, and it soon became noticeable that the piece was rising higher each time and

settling back less after each trial. Successive stages of this rising are shown in Fig. 39, *b*, *c*, *d*. Finally, it worked up till it stood directly on the posterior end (*e*), and then the next contraction caused it to fall over of its own weight and come down right side up (*f*). The sticky mucous secretion at the posterior end was undoubtedly what held the piece up after each successive trial. This behaviour, as described, was uniform in all the trials.

The behaviour of these pieces brings out several points of importance. First, it is to be noticed that no trace of the typical spiral righting reaction is to be seen; yet, on the other hand, we find the pieces bending strongly towards the cut side when first inverted, which is just the effect which would

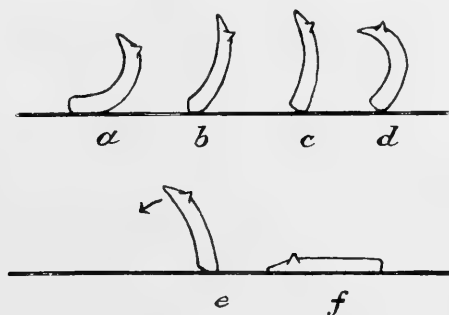


FIG. 39.—Diagram showing the method of righting adopted by one of the pieces shown in Fig. 38.

be produced by the lengthening of the stimulated side in the normal righting reaction, provided, as actually obtains in this case, there was no opposite side to keep the long axis of the piece straight. Thus we get precisely the result which would be expected if the view given of the mechanism of the reaction is the correct one. Another fact that is brought out by this experiment is the apparent adaptation shown. When the animal is unable to give the usual reaction for righting itself it very quickly reacts in an entirely different way, but attains the same end result.

A worm was cut so as to give a piece of the form shown at A in Fig. 40. This piece was placed in an inverted position

and its reactions observed. Evidently, so far as injury of the mechanism by the operation is concerned, such a piece is in essentially the same condition as the pieces described in the previous experiment. It has only one complete side of the body. The piece when inverted squirmed about considerably at first, but gave no indication whatever of the normal spiral reaction. In a short time the violent movements ceased, and a notch was noticed in about the middle of the uncut edge (cf. Fig. 40, *b*). This soon grew larger, and extended more and more towards the ends of the piece, as shown in

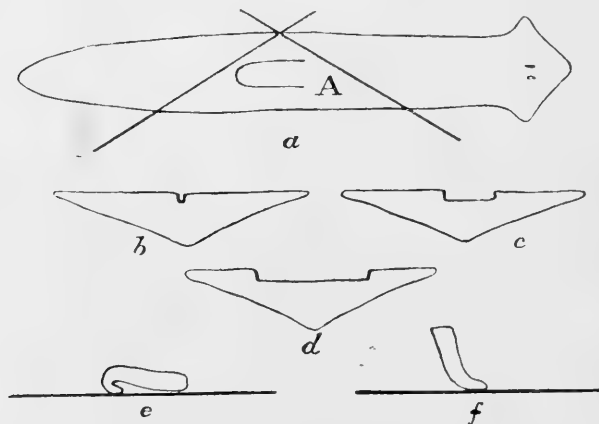


FIG. 40.—*a*. Operation diagram. Heavy lines indicate the cuts. *b*, *c*, and *d*. Successive stages in the righting reaction of the piece A of diagram *a*. *e* and *f*. Cross-sections through A at two successive stages in the righting process. See text for further explanation.

c and *d*. By close observation the cause of this appearance was found to be that the thin mobile edge was folding under and attaching its ventral surface along the bottom. A cross-section through the worm at this stage had the outline shown in *e*. As soon as a considerable portion of the edge had so folded under and become attached, the piece gave a series of strong contractions and literally “flopped” over the attached edge and came down right side up. A stage in this process is shown in cross-section in *f*. This behaviour was so peculiar, and at the same time precise, that the

experiment was repeated many times on this piece and on others cut in the same way. The same method of righting was always observed. After the first few times the turn is made in this way; it is done more quickly at each successive trial.

This experiment leads to the same conclusion regarding the mechanism of the righting reaction as did the previous one. It affords another and more striking example of regulation in reactions. The piece attains the end (normal position) by a reaction which it undoubtedly never had occasion to practise before.

Isolated longitudinal halves of the body react in the same way as did the piece described in the preceding experiment. They right themselves by folding under the edge, and then, by violent contraction, drawing the rest of the body up over it. There is no trace of the spiral righting reaction.

A specimen cut in the manner shown in Fig. 41 shows a

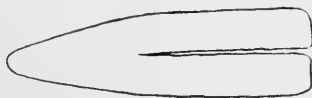


FIG. 41.—Operation diagram (see text).

very peculiar righting reaction. When placed dorsal side down the portion posterior to the median longitudinal slit immediately gives the spiral righting reaction, and drags the two passive anterior pieces over. The process is slow but very characteristic, so that there is no doubt of the nature of the reaction. This shows that in that part of a single piece of a worm where the necessary mechanism is present we get the spiral righting reaction, while in other parts it does not appear.

The same point can be brought out by splitting a worm longitudinally from the posterior end up to a point near the head. The complete anterior part of such specimens gives the normal spiral reaction, while the posterior parts remain passive so far as this reaction is concerned.

A considerable number of different experiments were per-

formed for the purpose of testing the righting reactions after operations, but since none of them bring out anything different in principle from the results already given, they will not be reported here. But it may be said in general, that all the experiments gave the same results with reference to the mechanism of the reaction, namely, that so long as the mechanism described above was intact the typical spiral reaction was given; when this mechanism was destroyed or injured the reaction was not given, but the animal, if it righted itself at all, did it by a different method.

When the animal falls freely in the water the righting reaction is induced because the ventral surface is no longer in contact with a solid. There is no reason for thinking that the mechanism of the reaction in this case is any different from what it is when the animal is placed in an inverted position on the bottom. The direction in which the spiral is thrown in the case of the falling animal is probably determined by slight differences of pressure on the two sides of the body.

c. Summary.—The flat-worm is positively thigmotactic on its ventral surface, and negatively thigmotactic on its dorsal surface. As a result of this it gives a characteristic righting reaction whenever the normal relations of either surface are changed. This righting reaction consists in throwing the body into a spiral in such a way as to bring the ventral surface of the anterior end down into contact with a solid (in all cases except when the animal is dropped into free water). The anterior end starts gliding and unwinds the spiral, thus righting the whole body. The thigmotactic reaction may be modified by chemical and other stimuli. All the evidence shows that the spiral righting reaction is due to a lengthening of the side whose dorsal surface is in contact with a solid, while the other side of the body keeps the long axis straight. The direction of the turn in the spiral is determined by the side of the body which is in contact with the solid. This reaction is thus seen to be closely related to the negative reaction to mechanical and chemical stimuli, so

far as mechanism is concerned. Cut pieces, in which the normal mechanism for the righting reaction has been destroyed, right themselves in various ways, thus showing a sort of regulation in reactions.

IV. Electrotaxis.

In view of the sharp and precise reactions of planarians to other stimuli, it was thought that they would furnish excellent objects for the study of electrotaxis, but unfortunately this is not the case. Their reactions to the constant current are not clear-cut, since the specimens become wholly or partially paralysed in a very short time after the current begins to act, and as a consequence the reactions become feeble and indistinct. For the sake of completeness, however, and since some facts of importance are brought out, the experiments on this subject will be briefly reported.

a. Methods.—The following methods were used:—The constant current used was obtained from the general lighting circuit of the University, and reduced to the proper intensity by interposed resistance. This apparatus for getting the current I have described fully elsewhere (: 00, : 01), so that it need not detain us here. In the circuit a rheostat was inserted for regulating the strength of the current. Ordinary unpolarisable brush electrodes were used. The specimens were placed either in a trough with clay ends, to serve as poles, and with paraffin sides of 5 mm. depth, or else on a slide under a cover supported by several layers of moistened filter-paper. These filter-paper ends then serve as the poles of the preparation, the brushes of the electrodes being laid upon them. The layer of water in which the specimens were in this sort of a preparation was approximately 2.5 mm. in thickness. Identical results were obtained by both the trough and the filter-paper method, but since the latter is the neater and generally more satisfactory method, it was used almost entirely in preference to the trough.

b. Results.—The typical result of the action of the current on specimens in such a position that the long axis of

the body is approximately at right angles to the direction of the current, may be described first. If a number of specimens are gliding about at the normal rate, and a current of from weak to medium intensity is made through the preparation, the first reaction of all the specimens is to stop their forward motion, turn towards the kathode, and start crawling very slowly towards this pole. The orientation towards the kathode is at the first trial usually rather precise. The whole animal gets squarely into line with the current and moves slowly towards this pole. While the current is acting the anode end of the body, in this case the posterior end, remains

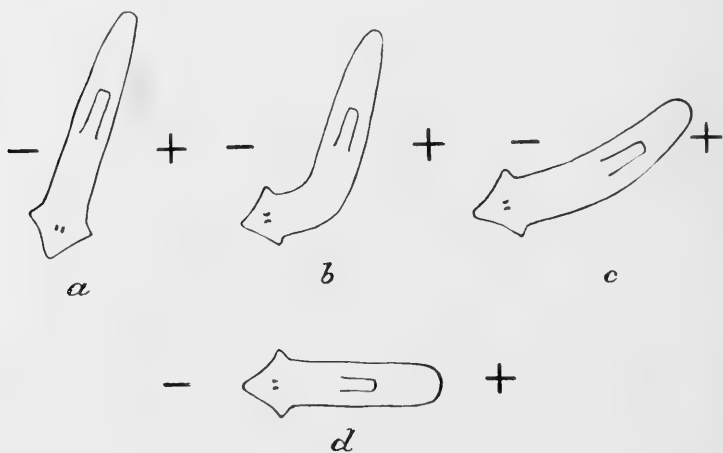


FIG. 42.—Diagram showing the typical electrotactic reaction of *Planaria*.
a. Position at the moment of making the current. *b*, *c*, and *d*. Successive phases of the reaction.

rather strongly contracted, presenting the same appearance as when mechanically stimulated. Movement occurs only for a short time after the current begins acting. The worm soon comes to rest, and further stimulation serves only to cause contraction of various parts of the body without producing any progressive movement. The successive stages of the first typical reaction to the constant current are shown in Fig. 42.

In succeeding experiments on a given individual, and in many cases with the very first experiment, the reaction is

much less pronounced. The animal in the transverse position, at the moment of making the current, will simply turn the anterior part of the body somewhat toward the kathode and then stop. Reversal of the current causes the head to swing a short distance towards the new kathode, and then stop again. The orientation becomes less and less precise the longer the current acts. The position most frequently taken by a specimen after it has been submitted to the action of the current for a short time is shown in Fig. 43, where it is seen that the orientation of even the anterior end is not very precise. In all such cases the specimen remains perfectly quiet after the first turn towards the kathode until the current is reversed or broken.

The behaviour described is that which is typical for currents of medium to fairly weak intensities. With very weak currents no striking effect is produced. With a current

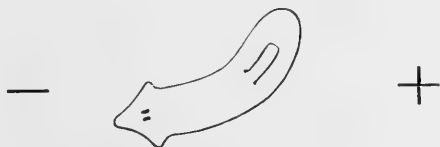


FIG. 43.—Diagram showing partial orientation of Planaria to the constant current.

which is just strong enough to cause a general movement of *Paramœcium* towards the kathode, the only effect on a planarian gliding at right angles to the current is to cause in some cases a very slight turn of the head towards the kathode at the moment of making. The specimen does not stop the gliding movement, and is not forced into any orientation, but may give a slight turning reaction, which changes its course from one squarely at right angles to the current to one turned a little diagonally towards the kathode. In many cases such a current produces no effect whatever. With very strong currents the planarian stops at the moment of making, jerks the anterior end around towards the kathode more or less, and then curls up into the form shown in Fig. 44, as a result of very strong contraction of the ventral longitudinal

musculature, and dies. I have never been able to produce disintegration on the anode side with any current strength at my disposal except in a single case, where disintegration began in the region just behind the pharynx in a specimen strongly curled up in the way described.

In case the long axis of the planarian is parallel with the



FIG. 44.—Diagrammatic side view of a planarian subjected to the action of a very strong constant current.

direction of the current, and the head is towards the kathode at the moment of making, with a perfectly fresh specimen the effect is to cause a cessation of the gliding movement and a change to a very slow crawling. The direction of the movement is not changed. There is a well-marked contraction of the anode (posterior) end of the body. The reaction of the animal in this position is shown in Fig. 45, *b*. Very weak currents have either no effect on a specimen in this position or else may cause a very slight contraction of the ventral longitudinal fibres mentioned above.

When the long axis of the body is parallel to the direction of the current, and the head is towards the anode at the

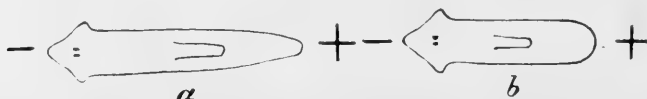


FIG. 45.—Diagram showing the electrostatic reaction of *Planaria* when the long axis of the body is in line with the current direction, and the head is towards the kathode. Contracted portions are indicated by heavy lines.

moment of making, the effect of a current of medium intensity is to cause the gliding movement to stop. At the same time there is a very definite contraction of the anode (head) end of the body. As the current continues to act the specimen begins to squirm about, and very soon gets out of line with the current. Then the anterior end is turned towards the

kathode slowly, and this process may be continued until complete reversal is brought about and the animal comes to lie again in line with the current, but with the anterior end now directed towards the kathode. This reversal into the usual orientation is the typical reaction for fresh specimens at the first trials of the current; it is shown in Fig. 46. In case the specimens have been under the action of the current for some time, there is no reversal of the position. The specimen

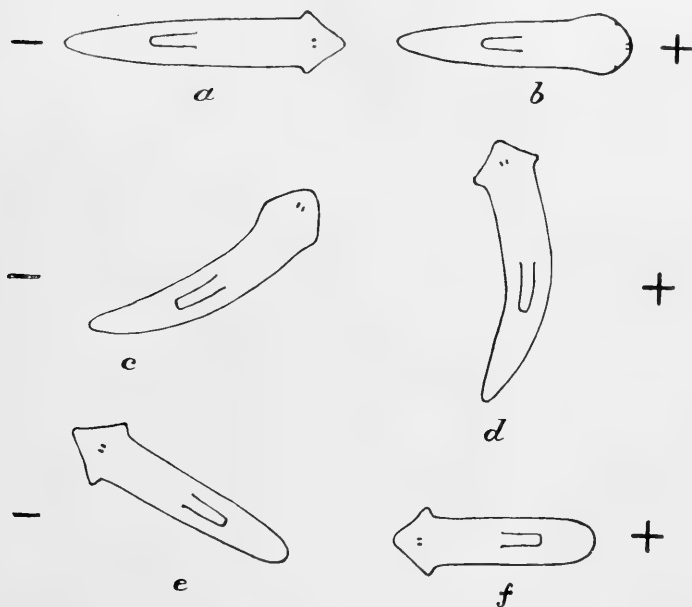


FIG. 46.—Diagram showing the electrostatic reaction of *Planaria* when the long axis of the body is in line with the current direction, and the head is towards the anode. Contracted portions are indicated by heavy lines.

simply remains in the same position and contracts strongly at the anode (head) end of the body.

Strong currents have the same effect as described in the preceding experiment. Very weak currents either have no definite effect, or else cause a slight jerking back of the head, and turning a little to one side at the moment of making.

After the animals have become partially paralysed by the

action of the current, the nature of the contractions and relaxations of different parts of the body can be very clearly seen, and since these are the most significant features of the animal's reactions to the electric current, they may be described a little more fully. These reactions for the three chief positions are shown in Fig. 47. The essential features are contraction of the anode end of the body when in line with the current, and convexity on the anode side when at right angles. Besides this there seems to be some slight expansion at the kathode end of specimens in line with the current, but this appearance is not constant. Reversal of the current in these paralysed specimens causes contraction at

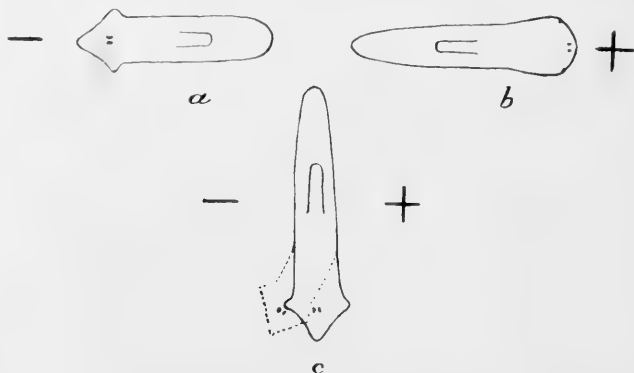


FIG. 47.—Diagram showing the contractions caused by the current with the body in the three principal positions.

the new anode end or bending towards the new kathode. On breaking the current the contracted portions relax.

c. Mechanism of the Reactions.—It will be seen from the figures, and the account which has been given of the responses to the electric current, that there is an apparent anomaly in the behaviour. The specimen contracts always at the anode end of the body, but apparently not on the anode side of the body. I believe that the explanation for this apparent difference in behaviour is to be found in the structure of the animal, and in a peculiarity in the action of the constant current which has been noted in another case. When the animal is in line with the current the contraction

observed at the anode end is, as shown by the form taken by the part reacting, a contraction of the longitudinal muscle-fibres, while the fibres of the circular and transverse system are relaxed. In other words, the current only affects those fibres which bear a definite orientation with relation to direction of its flow, viz. those which are parallel with it. Now it has been shown in an earlier part of this paper that in the ordinary negative reaction the turning away from the stimulus is produced by a contraction of the circular, transverse, and dorso-ventral fibres (principally the circular) on the side stimulated. Evidently when the animal is at right angles to the direction of the flow of the current the only muscle-fibres in the body whose longitudinal axes are in line with the current are the fibres of the circular and transverse systems. Unless it is assumed that the current acts differently in one case from in another there is no apparent reason why, when the animal is in the transverse position, the fibres which are in line with the direction of the current on the anode side of the body should not contract. If the fibres fulfilling these conditions as to location and orientation (the circular system) do contract, they will cause the anterior end to be turned towards the kathode and the anode side to become convex,—in other words, produce the actually observed result. The fibres of the longitudinal system should not be affected, and there is no evidence that they are. This explanation assumes that the current produces its effect by directly causing the contraction of properly oriented muscle-fibres, possibly, or even probably, without relation to the stimulation of any sense-organs of the animal. Or, to put it in another way, the responses according to this view might not necessarily be reactions of the organism at all, in the sense of being something that the animal does after receiving and transforming a stimulus, but are direct effects of the stimulus acting on the motor organs. It has doubtless occurred to the reader that another explanation is possible for these reactions, namely, that they are in no way essentially different from what would be produced if the animal were given strong mechanical

stimuli on those parts of the body which are nearest the anode in the several positions. In other words, the constant current, from the standpoint of the planarian, produces the same effect on the anode side or end of the body that a strong mechanical stimulus applied in the same place would.

Which of these two views is the correct one the planarian does not show clearly. Yet there is some inferential evidence which makes it seem probable that the first view as to the cause of the reaction is the correct one, viz. that the current produces direct contractions of muscle-fibres oriented in line with its direction. The evidence for this view is as follows :—

(1) In the case of specimens which have been for some time under the action of the current, and are, as has been mentioned, almost completely paralysed, the essential features of contraction on the anode side or end can still be produced by a fairly weak current. At the same time it takes a very strong mechanical stimulus to get any reaction from these pieces, indicating that their sense-organs are almost completely paralysed, and their general sensitivity gone. If the current acts merely as a stimulus qualitatively like others which produce the same reactions, it is not apparent why it should be effective in weak intensities when another stimulus fails in strong intensity. If it acts directly on the muscles we should expect that it would be capable of producing an effect after the general sensory functions had been lost. (2) The contractions produced by the current are sharply localised, i. e. they involve only a certain definite part of the body whether the current is strong or weak (within certain limits); whereas mechanical stimuli applied to the same places with an intensity sufficient to cause the same definitive reaction will also cause a marked general response of the whole organism. This is just what would be expected if the current affects only the muscles oriented in line with it and lying at the anode pole of the worm. (3) By analogy with other forms—for example, the Protozoa—it would be expected that the current would produce some other effect than that of an ordinary stimulus applied at the

same point. In the case of the Infusoria the current causes an entirely different reaction from that produced by any other known stimulus.

For these reasons, then, I am inclined to think that in the case of the flat-worm the current affects certain definitely oriented muscle-fibres directly, and by this means produces in the main the characteristic reactions. That the current does not also stimulate the sense-organs, and so act like other stimuli applied to the same places, I am not prepared to say, but it seems probable that the phenomena observed are not primarily caused by such action.

It has been brought out by inference that the cilia play no part in the electrotactic reaction of planarians. This is the true state of the case. The current in any intensity sufficient to cause the definite reactions stops immediately, so far as I have been able to observe, all ciliary movement. The evidence for this is twofold. First, all gliding movement stops in effective currents; and second, by direct observation of specimens crawling ventral side up on the surface film no ciliary currents can be observed while the electric current acts. This result is of interest in connection with the reactions of the rhabdocœle *Stenostoma leucops*, O. Schm. This form, which normally moves freely through the water by the activity of its cilia, reacts to the electric current in essentially the same way as do the Infusoria (cf. Pearl, :00). That is to say, the cilia on the kathode half of the body take a reverse position when the current is made, and their effective stroke is towards the anterior end. The different relations of the cilia in different positions of the body are shown in Fig. 48. This relation of the ciliary beat, coupled with the form of the body, causes, as a mechanical necessity (cf. Ludloff, '95), the animal to orient with the anterior end towards the kathode. This method of reaction of *Stenostoma* I worked out by precisely the same methods as I used in a previous study of the electrotaxis of the Infusoria (:00). This reversal of the position of the cilia as a result of the action of the current has hitherto been observed only in

the Infusoria, and to find the same thing in a multicellular organism is a matter of considerable interest. It is outside the scope of the present paper to discuss the relation of this result to current theories of electrotaxis, as I hope to be able to do in a later paper, but it may be said that this furnishes another strong piece of evidence that in the case of these lower organisms the current does not cause the observed reactions in any way comparable to that in which a mechanical stimulus causes a reaction, i. e. by furnishing a certain "sensation." On the contrary, the current acts as a physical force on a structure organised in a certain way.

Experiments on the electrotactic reaction of cut pieces of

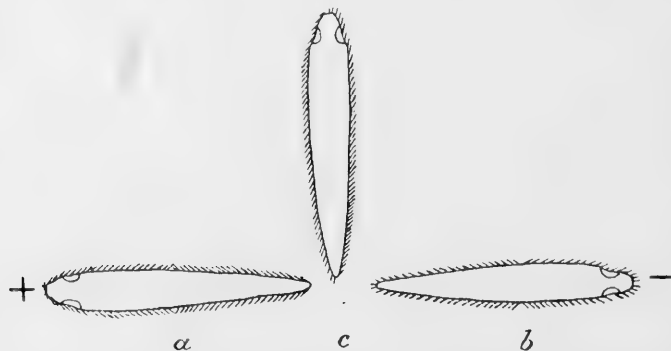


FIG. 48.—Diagram showing the electrotactic reaction of the rhabdocoele, *Stenostoma leucops*, O. Schm.

planarians have been tried in considerable numbers, but with, on the whole, unsatisfactory results. Anterior pieces resulting from transverse cuts are the only ones from which I have been able to obtain any constant results. Such pieces react like the normal animal in every way. Posterior pieces from transverse cuts show the contractions on the anode side and ends in a slight degree, but there is no constant production of orientation. Specimens slit longitudinally in the middle line from the posterior end nearly to the head react essentially like a normal specimen, although much more weakly. I have observed in one case fairly precise orientations of such a specimen. From specimens slit longitudinally

in the middle line from in front backwards I have never been able to obtain any definite results. They simply squirm about in an aimless way for a moment when the current is made and then become quiet, and remain so while the current passes. The direction in which the current is flowing makes no difference in their behaviour. All operated specimens become very quickly paralysed by the current.

d. Summary.—The constant current very quickly paralyses planarians: Its specific effect is to cause a contraction of the anode side or end of the body. This produces in the case of fresh specimens a well-defined orientation, with the anterior end towards the kathode. All progressive movement after the making of an effective current is by the crawling method, the cilia being stopped or very greatly slowed in their beat. The electrotactic reaction, so far as the attainment of orientation is concerned, is essentially the same as the negative reaction to mechanical stimuli. In the rhabdocœle *Stenostoma leucops* there is found to occur a reversal of the cilia on the kathode half of the body, such as occurs in the case of the Infusoria.

V. Reaction to Desiccation.

A series of experiments was performed to determine the reactions of the animal on drying. This is an environmental condition which planarians probably have had to meet with relative frequency in the course of their history as a species, and it is a matter of interest to determine whether they have any method of reacting which protects them from it.

Experiments were first performed in the following manner:—Specimens were taken from the aquarium dish on the point of a scalpel or a spatula, and lightly touched to a filter-paper for a moment to remove any adherent water, and then laid upon a dry surface—either glass or paper. The behaviour was usually as follows:—The worm would curl up closely and thrust the head under the body, as shown in Fig. 49. The purpose of their behaviour seems to be to get the body into as small space as possible, and especially to keep

the head from drying. At fairly frequent intervals the animal straightens out and extends the head in front as far as possible, and makes "feeling" movements. It is then withdrawn, and the animal curls up again. After the drying has proceeded for some time the most characteristic feature of the whole reaction appears. This is a lengthening of the posterior part of the body to its fullest extent. The posterior end then attaches itself to the surface, and strong waves of contraction, like those in the crawling movement, pass over the body from the posterior end forward. No progressive movement is made, but backward crawling is evidently attempted, and is only prevented by the dry surface which the animal is on. There may be considerable variation in the first part of the reaction with regard to the curling up; this may appear or may not, but the attempted backward crawl-

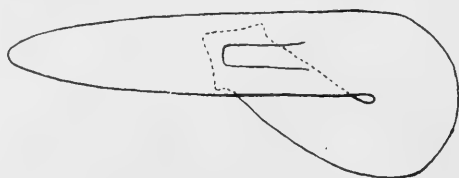


FIG. 49.—Diagram showing the reaction of *Planaria* to desiccation.

ing movement of the posterior part of the body I have found to be a constant feature in the experiments which I have performed. When the dorsal surface of the worm becomes dry all movement ceases. If quickly put back into the water the worm will usually recover completely, even though all movement has ceased in the air.

If the worm is put on a slide in the centre of a small area which has been wet, but on which there is no standing water, it will squirm about and extend the head frequently, as in the last experiment. If the head goes outside the wet area it is very quickly jerked back, and the specimen gives the negative reaction, i. e. turns away from the side stimulated. The attempted backward crawling occurs in this case just as in the others, a short time before the dorsal surface dries off.

It is to be noted that there is never any actual progressive

movement of a specimen in the air. If a specimen is placed on very wet filter-paper it is not able to progress unless water is kept constantly dropping on it from above, so that it is at any time surrounded by a layer of water. On account of this lack of ability to move when out of water, there is no true hydrotaxis in the sense of movement towards water.

As has been mentioned before, specimens placed on a dry surface dorsal side down do not show the righting reaction.

To sum up, it is found that planarians, when removed from the water and subjected to a process of drying, are unable to make progressive movements. At a certain stage in the drying process they attempt to crawl backwards—a form of movement which, under certain circumstances, might get the animal back into water. On meeting a dry surface with the anterior end the animals give a well-marked negative reaction. The animal does not give the righting reaction on being inverted on a dry surface.

On the whole, the general behaviour when subjected to drying is purposeful; that is, it would tend to prevent the animal ever becoming dried up under natural conditions. There is nothing in the behaviour of planarians to indicate how the change from aquatic to terrestrial life could be brought about. The fresh-water Triclad, so far as I have observed them, never leave the water and crawl up into the air above the surface film as some other forms do.

VI. Rheotaxis.

A large number of experiments were performed early in the course of the work with various sorts of devices to determine whether the animal showed any distinct reaction to currents in the water, but without success. Streams of water from a pipette, currents made by filling the tube of the diffusion apparatus described above (pp. 661, 662) with water and blowing into it, and other methods gave no results. If the currents were made with sufficient force to threaten dislodgment of the animal from its hold on the bottom it would stop moving and contract longitudinally, and thus attach itself

more firmly to the substrate. Weaker currents caused no effect whatever. I was inclined to believe that the longitudinal contraction and the gripping of the bottom were the only rheotactic reactions which the organism exhibited. It was found later, however, that there was a very precise rheotactic reaction of a different character. In the course of the experiments on reactions to localised chemical stimuli by the capillary tube method, it was discovered that by using a tube with a relatively large opening (from $\frac{1}{4}$ to $\frac{1}{2}$ mm. in diameter) and letting the ordinary tap-water in which the animals were flow out of it, by its own weight, a current of just the right intensity to cause a positive reaction could be produced. The animals would turn very sharply towards the source of such a current, the reaction being evidently the same as that given to other weak stimuli (chemical and mechanical). This reaction is localised in the same way as the usual positive reaction. It is given only when the current is directed against the head or anterior part of the body.

It is thus seen that the planarian is positively rheotactic to very weak currents, the form of the reaction being precisely the same as that given to other weak stimuli. It seems very doubtful if this reaction is of any importance in the normal activity of the animal.

G. GENERAL SUMMARY AND DISCUSSION OF RESULTS.

As was stated earlier in the paper, the problem with which this study deals is the analysis of the behaviour of the common fresh-water planarian. The movements and reactions to all the more important stimuli, with the exception of light and heat, have been described and analysed into their component factors in the body of the paper. It is believed that it is of the greatest importance to have as complete and detailed an account of the various activities as possible, and as a consequence full details have been given in the case of each subject treated. Since this method of treatment necessarily makes the account of considerable length, it has a tendency to obscure the general and significant results in a

mass of detail. It is desirable, then, to state clearly at the end the important general facts which have been brought out by this study, and to discuss to some extent their significance. In this place I shall state the results in a categorical manner, making no attempt to indicate the evidence on which the conclusions are based. This will avoid needless repetition.

1. The locomotor movements of *Planaria* are of two sorts, gliding and crawling. The gliding movement is produced by the beating of the cilia on the ventral surface of the organism. It is by far the most usual method of locomotion. For its production it is necessary that there be a layer of sticky, mucous slime between the ventral surface of the body and the substrate. In this slimy secretion the cilia beat and so propel the animal (cf. pp. 544 and 545). The organism never moves freely through the water without some sort of mechanical support. The rate of the gliding is changed by the action of various agents, such as light, chemicals, electricity, etc. Its direction is always forward.

The crawling movement is produced by strong longitudinal waves of muscular contraction passing over the body from the anterior to the posterior end. It is more rapid in rate than the gliding. It appears only after strong stimulation of the organism, and its purpose is evidently to get the animal quickly away from harmful stimuli. Its direction may be either forward or backward.

Periods of movement alternate with periods of rest in the course of the animal's daily activity. When at rest the flatworm is in a condition of relaxation and generally lowered tonus, corresponding to the condition of a higher organism in sleep. The causes which induce the coming to rest are—*(a)* a more or less fatigued condition of the organism. This is the primary cause; without it the other causes are ineffective. *(b)* A relatively low intensity of light. *(c)* Roughness of the substrate. This brings the body into a position such that its different parts form angles with one another, and causes the animal to come to rest as the result of a reaction which I have called *goniotaxis* (p. 562). *(d)* Certain chemical con-

ditions. As a result of the action of some one or all of these above-mentioned factors, collections or groups of planarians are frequently formed.

Planarians which have been injured by operative procedure move comparatively little during the course of regeneration, thus showing a sort of regulation or correlation between behaviour and morphogenetic processes (pp. 573, 574).

2. There are two principal qualitatively different reactions to stimuli, the positive and negative reactions.

The negative reaction is given in response to strong unilateral stimulation of the anterior portion of the body. It consists essentially in a turning of the head away from the side stimulated. It is brought about by the extension of the body on the side stimulated. This extension is produced by a contraction of the circular, dorso-ventral, and transverse systems of muscle-fibres. The purpose of the negative reaction is evidently to get the organism away from harmful stimuli.

The positive reaction is given only in response to weak unilateral stimulation of the anterior portion of the body. It is essentially a turning of the head towards the source of the stimulus. This reaction is one of considerable precision, bringing the anterior end into such a position that it points in most cases exactly towards the source of the stimulus. The turning is brought about by the contraction of the longitudinal muscle-fibres of the side stimulated. The evident purpose of the positive reaction is to get the animal into regions of beneficial stimuli.

3. Whether the negative or the positive reaction shall be given in response to a particular stimulus depends primarily on the intensity of the stimulus, and secondarily on its location. Neither reaction is given unless some part of the body in front of the pharyngeal region is stimulated. The negative reaction is given only in response to stimuli above a certain intensity (strong stimuli). This relation between intensity of stimulus and form of reaction holds for both mechanical and chemical stimuli.

4. The reactions of *Planaria* to a variety of chemicals, including representatives of several of the most important chemical groups, were studied. It was found that to a weak solution of any substance, regardless of its chemical composition, the organism gave a positive reaction identical with the positive reaction to mechanical stimuli. To strong solutions of the same substances (with a single exception, see p. 657) the organisms responded by a negative reaction identical with that caused by strong mechanical stimuli.

Planaria does not orient itself to a diffusing chemical in such a way that the longitudinal axis of the body is parallel to the lines of diffusing ions. Its reactions to chemicals are motor reflexes identical with those to mechanical stimuli. The positive reaction is an orienting reaction in the sense that it directs the anterior end of the body towards the source of the stimulus with considerable precision, but it does not bring about an orientation of the sort defined above.

5. Several important features in the normal behaviour of the flat-worm are found upon analysis to have their explanation in the positive and negative reactions to mechanical and chemical stimuli.

The method by which the organism gets its food is simply a special case of the positive reaction. From substances which serve as food for the planarians, various juices diffuse into the surrounding water. When the planarian meets any of these diffusing substances it gives the positive reaction,—that is, turns in the direction from which the stimulus comes. The food substance acts as a weak chemical stimulus, to which the animal reacts in the same way as to all other weak chemicals.

The direction of the planarian's movement, and its behaviour with reference to obstacles in its path, are usually determined by its reactions to mechanical stimuli.

The behaviour of the organism with reference to the surface film is determined by its reactions to mechanical stimuli.

6. Strong stimulation—either mechanical or chemical—of

the posterior portions of the body induces the crawling movement. This is to be regarded as the specific reaction of this portion of the body. Weak stimulation of the same region causes local contraction at the point stimulated in the case of mechanical stimuli, while weak chemical stimuli applied to this region are ineffective.

7. The ventral surface of the body of *Planaria* is strongly positively thigmotactic, and the dorsal surface is negatively thigmotactic.

8. When the organism is placed in an inverted position it performs the righting reaction. This reaction consists in a turning of successive parts of the body about the longitudinal axis through 180° . During the process the animal takes the form of a spiral. The anterior end is brought into the upright position first. On analysis the righting reaction is found to be a special case of the reaction to strong stimuli (the negative reaction). It is brought about by an extension of one side of the body, while the other side maintains its original length (pp. 676—679). The reaction is given whenever the ventral surface is removed from a solid or the surface film of the water.

9. To the constant electric current *Planaria* reacts by turning the anterior end towards the kathode. Complete orientation and movement towards the kathode may occur. The turning towards the kathode is brought about by an extension of the anode side of the body. The current causes a contraction of muscular elements whose long axes are parallel to the direction of the current (pp. 690—693). The current very quickly paralyses planarians on which it acts.

The rhabdocœle *Stenostoma leucops* orients to the current with the anterior end towards the kathode, and moves towards this pole. This orientation is brought about by changes in the positions and consequent effective beat of the cilia, exactly like those which occur in the case of the ciliate Infusoria. Cilia, on the portions of the body directed towards the kathode pole, take on reversed positions.

10. All the normal reactions to stimuli are of the nature of

reflexes, more or less complex. What the animal will do after a given stimulus, or in a given situation, can be predicted with reasonable certainty. There is, however, some variation in the behaviour, depending on the physiological or tonic condition of the individual at the time of stimulation. Thus a stimulus sufficiently weak to induce the positive reaction in one specimen may cause the negative reaction in another; or at different times the same individual may show different reactions—either the positive or negative—to the same stimulus.

11. Psychological Position of Planaria.—The objective psychological position of any organism is evidently determined by the relative simplicity or complexity of what it does. With a view of determining what the position of Planaria in the psychological scale is, it may be well to make a catalogue of the things which it does in the course of its ordinary existence.

The animal performs the following acts:

a. It moves progressively by two methods, a ciliary motion and a muscular motion.

b. It turns, by a complex of simple reflex acts, towards all weak stimuli investigated.

c. It turns, by another set of simple reflex acts, away from all strong stimuli investigated.

d. It comes to rest in certain definite environmental situations.

e. When stimulated in a certain way it extends the pharynx and feeds.

f. When its ventral surface is removed from contact with a solid body (or the surface film), a reflex of essentially the same character as that of *c* brings this surface again into contact with the solid.

From these essential factors is composed a behaviour whose complexity one has only to study to realise.

The behaviour is thus seen to be, in the main, what may be characterised as reflex. It is very simple to say that an animal's activity is composed of a series of invariable reflex acts in response to stimuli, but I doubt whether the full

significance of such a condition is always realised. It implies that the animal as an individual "does" nothing in the sense that a man "does" things. It is moved about from place to place by its locomotor organs; it is put into certain definite and invariable relations to its surroundings by its reflex mechanisms. Considered as a whole, such an organism is a sort of shell to hold a series of mechanisms, each of which is independently capable of doing a certain thing, and in the doing produces some effect on the shell as a whole. We may perhaps get a clearer picture of what such a reflex existence means by considering for a moment what would be the effect if all a man's activities were composed of invariable reflexes, to be set off by the appropriate stimuli. Under such circumstances, whenever a man saw or smelled food he would have to go to it and eat it. Whenever anything touched him he would have to move in a new direction very closely related to the position of the object which touched him. Whenever he touched water he would have to take a bath, or perhaps drink till he could hold no more. During the day he would have to move always in a definite direction with reference to the sun, and so on *ad infinitum*. All he did would be definitely fixed and, in a sense, predetermined by the things about him.

It is apparent that the behaviour of *Planaria* is not thus entirely and purely reflex, because there is a certain amount of variation in it. As has been brought out in several places in the body of the paper, and in paragraph 10 of these conclusions, this variation in the behaviour is the result of the physiological condition of the individual. To put this in a more concrete form, we may say that a fatigued animal or an animal in a state of great excitation does not always react to a certain stimulus by the same set of reflexes as that by which a normal animal would react. Furthermore, there is a variation in the intensity of the negative reaction dependent upon the intensity of the stimulus producing it.

Another point in which the reactions of *Planaria* differ from what would obtain in the case of an organism whose

behaviour was composed of invariable reflexes is found in the behaviour following repeated strong stimuli applied to the anterior end (vide pp. 580, 581). In this case the organism shows an evident modifiability in reaction, for after giving for some time the ordinary negative reaction, and not thereby getting away from the stimulus, it finally turns directly towards the source of the stimulus. Again, in the righting reactions of pieces of the body we see entirely new forms of reaction appearing (pp. 680—683).

In order to give a concrete idea of the psychological position of *Planaria* it may be well to present in parallel columns the principal factors which make for simplicity in the behaviour on the one hand, and for complexity on the other hand.

Factors which tend to make the Behaviour Simple.	Factors which tend to make the Behaviour Complex.
A. Essential reflex character at the basis of all the reactions.	A'. Comparatively large number of qualitatively different general reactions.
B. General lack of modifiability of reactions.	B'. Marked qualitatively different reactions to differing intensities of stimulus.
C. Comparatively small number of qualitatively different reflexes composing the general reactions.	C'. Definite relations of reactions to location of stimulus.
	D'. Rather close dependence of reactions on the physiological condition of the individual. This brings about variation in the reactions.

The behaviour of *Planaria* is evidently much more complex than that of the Infusoria, as described by Jennings (loc. cit.). In the case of the Infusoria, all the factors A', B', C', D', which make the behaviour of *Planaria* so complicated, are nearly or quite absent; and in respect to C these organisms are at a much lower stage than *Planaria*. The Infusoria have practically but one purely reflex reaction to nearly all stimuli, and this reaction is not localised with

reference to the location of the stimulus. Again, the Infusoria do not show qualitatively different reactions to differing intensities of stimuli, as does *Planaria* to a marked degree. We thus see that *Planaria* stands considerably higher in the psychological scale than the Infusoria, and that the development is taking place along two main lines: (a) the higher organism reacts differentially with reference to the location and intensity of the stimulus; and (b) the physiological balance in the higher organism is much more delicately adjusted than in the lower, and as a consequence we see much more variation in the physiological condition. These variations in the physiological condition bring about variability in the reactions.

In the case of the ctenophore *Mnemiopsis leidyi* we have an intermediate stage between the Infusoria and *Planaria*. Here the animal reacts with reference to the position, but not the intensity of the stimulus. This condition, in which an organism reacts with relation to the position of a stimulus, and not to its intensity, must be for the individual a precarious one, because the animal must either go towards or away from all stimuli alike, whether good or harmful. Chances are theoretically equal that after each stimulus it may get a toothsome morsel of food, or, on the contrary, serve in that capacity itself. Further development beyond the point in the behaviour series where *Planaria* stands must be in the line of further differential reactions with reference to quality of stimulus. A beginning along this line is made by the planarian, and the process is carried a step farther in the case of *Gonionemus*, as recently described by Yerkes (*loc. cit.*).

12. Relation of Behaviour and Structure.—The reactions of organisms are evidently, in any case, very closely dependent on the structural relations of the given organism, and on the conditions under which it lives, i.e. its environment in the broadest sense. Thus we find the asymmetrical Infusoria, which live freely in the water and move about by means of cilia, all reacting in the same way, and the determinative

factor in the reaction is the asymmetry of the body (cf. Jennings, :00). Now Jennings has further found¹ that certain rotifers, which live freely in the water and move about by the activity of cilia in a similar way, and furthermore are asymmetrical in fundamentally the same way that the Infusoria are, react in essentially the same manner as do the Infusoria. Similarly, I believe that the general reactions method of the planarians may be found to be in the main the method by which all organisms presenting the same general structural relations and mode of life react. Only one example on which this conviction is based may be given here. In the case of such fresh-water molluscs as *Physa* it is apparent that the actual locomotor and sensory organisation is symmetrical in form, and furthermore these forms live in fresh water on the surface of solid bodies just as do planarians. Now I have found, in a series of observations not yet published, that in the case of several of these molluscs the fundamental scheme of reaction is like that in the planarian. They react in the same way with reference to the location and intensity of the stimulus, and these are the fundamental things. In fact, the general behaviour is strikingly alike in the two widely separated groups.

13. Purposive Character of Reflexes.—A fact which is strongly impressed on one working on the behaviour of an organism whose activities are largely reflex is the purposive character of these reflexes. They are so adjusted that in the long run they keep the animal out of danger, and get it into favourable conditions. In the flat-worm these two things are very well done in general by the negative and positive reactions. Of these two reactions it is easy to see that the positive is the more highly developed, in particular in the fact that it is much more precisely localised with reference to the position of the stimulus. We can see a reason for this in the fact that under the conditions of the planarian's life the

¹ Complete observations not yet published. For preliminary account see 'Science,' N. S., vol. xv, pp. 524 and 525; and Jennings, :01, in bibliography at the end of this paper.

getting of food is of far more importance in the struggle for existence than the avoidance of danger. This point has, however, been discussed earlier in the paper, and need not detain us here. The real problem is presented in the attempt to discover how any of the purposive reflex acts in the organisms arose. I see no reason for denying that many of them—such as, for example, the positive reaction which gets the animal its food—were developed by natural selection. There are other evidently purposeful reactions, however, with whose development it hardly seems as if natural selection could have had anything to do, since they cannot themselves be of selective value. This point has been well brought out in a recent paper by Morgan (:02, p. 281). I think a possible explanation of some of these may be found in their analysis into component factors, when it may appear that only a very few simple reflexes had to be formed by natural selection, and then all the reactions are built up from these. An example will make my meaning clearer. In the righting reaction of the planarian we have a fairly complex reaction which is evidently immediately purposeful. Yet we find on analysis that this reaction is at bottom nothing but a slight modification of the ordinary negative reaction, which might very well have been developed by natural selection. And thus it is with other reactions and pieces of behaviour. They are for the most part built up from a very few simple purposive reflexes. If we can get them subdivided and spread out, as it were, so that we can see what goes to compose them, we may find that our problem has diminished very much, and we shall have to deal with only a few factors where before there appeared to be many.

A difficult problem in purposeful behaviour presents itself when we find that new methods of reaction appear at once if the usual reaction is prevented. The best examples of this are found in the righting reaction of cut pieces of planarians. Here we find pieces of the body, in which the normal mechanism of the reaction has been destroyed, immediately reaching a certain end (the righting) by a method differing

entirely from any that planarians ever used before to attain the same end, so far as we have evidence. These phenomena have a considerable resemblance to such phenomena as the well-known regeneration of the lens from the iris in some Amphibia. It is not easy to see how such behaviour comes about, and natural selection helps us very little. The matter belongs apparently to the same class of phenomena as morphological regulations, and probably has ultimately the same explanation. What this explanation is we do not know.

14. Functions of the Nervous System.—The most important function of the brain is the preservation of the tonus of the organism. After its removal the general tonus rapidly diminishes, and on this account the positive reaction—which depends rather closely on the physiological condition—can be obtained only with great difficulty in such decapitated specimens. There is no evidence of the presence of special centres in the brain. The nervous system, as a whole, has its main function in the rapid conduction of impulses.

15. Subjective Psychic Attributes.—One of the principal questions which forever recurs with regard to work on animal behaviour is, does the animal possess consciousness? Now although it has been shown what the component parts of the activities of the planarian are, yet it cannot be said, as it seems to me, that the planarian does not, or, on the other hand, that it does, possess consciousness. All that any such an organism ever has done in the past, or ever will do in the future, cannot tell us whether it was conscious in the doing or not. Any "objective criterion" of consciousness does not exist. Furthermore, whether consciousness is or is not present in any given case is not, in any event, the greatest concern of the physiologist, who rests content with the objective explanation of how results are brought about, regardless of what the animal is thinking about the matter. On this subject Claparède (: 01, p. 24), in concluding an interesting and valuable discussion, has said, "*À la question ; les animaux sont-ils conscients ? la physiologie—et même la psychologie en tant que cette science est explicative—doivent*

done répondre non seulement, 'Je l'ignore,' mais encore, 'Peu m'importe'!" With this standpoint I am in thorough accord.

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On the Diplochorda.¹

IV. On the Central Complex of *Cephalodiscus dodecalophus*, McI.

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With Plates 32 and 33.

INTRODUCTION.

IN the following description a number of the organs involved already possess a plurality of names, arising from the fact that various observers have recognised differing homologies, and emphasised them in the nomenclature. Thus the "buccal shield" of *Cephalodiscus* is also commonly termed the "epistome," "oral disc," and "pre-oral lobe." In the case of the two canals opening from the cavity of the buccal shield to the exterior there is the same difficulty. From homology with *Balanoglossus* they are termed the "proboscis pores," although the buccal shield has never been termed the proboscis; and they are more than pores, being of the nature of definite canals. As this work indicates an even closer structural resemblance to *Balanoglossus* than heretofore recognised, it would be well to retain as far as possible the nomenclature indicating this relationship; hence the terms collar cavities and trunk cavities are retained, whilst the term pre-oral canal is used as a synonym of proboscis pore with its attendant canal. The term subneural gland is retained for the "noto-

¹ Read before the Royal Society of Edinburgh, May, 1901.

chord" of Harmer, with its homology to the similarly named structure in *Balanoglossus*. Lastly, the terms pericardial sac, glomerulus, and ectodermal pit are adopted for organs in *Cephalodiscus* which seem to be homologous with similarly named organs in *Balanoglossus*.

In the region lying between the buccal shield and the collar of *Cephalodiscus* are several important organs, the exact relationships of which have not previously been fully determined. This area may be described as the central complex, a convenient term already applied to the same region in *Balanoglossus*. In this region there are externally the ectodermal pit, the pre-oral pores, and the central nerve-mass; internally are the subneural gland, certain important blood-vessels, the pericardial sac, and the mesenteric walls of the pre-oral and collar cavities, together with the glomerulus and muscular strands. The general outlines of the subneural gland and the pre-oral and collar cavities have been indicated by previous workers (McIntoshs, Harmers) and by myself, but a detailed examination by carefully orientated and serial sections has brought to light some interesting new facts.

We may describe the organs under the following headings:

1. Ectoderm and nervous system, ectodermal pit, pre-oral pores.
2. Subneural gland and pharynx.
3. Pericardial sac, pre-oral cavity, pre-oral canals, glomerulus, collar cavities, and blood-vascular system.

1. Ectoderm.—Figs. 1—8 all illustrate the condition of the ectoderm in this region. Ventrally the ectoderm on the buccal shield consists of long narrow epithelial cells with numerous unicellular glands, which form a buccal gland as described by McIntosh (6). This epithelium is not shown here. Dorsally it consists of columnar epithelial cells with a very definite cuticle. In the region of the central nerve-mass the inner ends of the cells are seen to pass downwards as delicate fibres, terminating in peculiar conical ganglion-cells (figs. 1 and 2). At their base these ganglion-cells give off other delicate fibres running forwards and backwards.

These fibres make up the main nerve-mass lying over the subneural gland; they are seen in transverse section in figs. 6—8, and in longitudinal section in figs. 1—5. Forwards they run along the dorsal surface of the buccal shield, and backwards they branch outwards to form the two lateral cords; hence, in longitudinal sections such as figs. 1—5, the nerve-fibres appear to terminate abruptly backwards against the wall of the pharynx, which here is in contact with the dorsal ectoderm. The same remark applies to the dorsal blood-sinus.

Immediately in front of the central nerve-mass is seen a pit or depression, the ectodermal pit (fig. 1). This pit lies exactly over the apex of the subneural gland, and extends transversely in a slightly crescentic form. At the outer ends of the crescent open the pre-oral pores, which are situated at the posterior termination of the pre-oral cavity, at the level of the central complex. The ectodermal pit, therefore, represents the line of division dorsally between the head or buccal shield and the collar, as do the “epidermistache” of *Balanoglossus* and the epiblastic pit of *Actinotrocha* (formerly termed the neuropore).

It is well to notice in dealing with this part that the nerve-mass is co-extensive with the collar, as this part is considerably narrower in the dorsal region; and further, that the subneural gland lies entirely in the collar area. At first sight one is inclined to suppose, reasoning from prior knowledge of *Balanoglossus*, that the two collar cavities are produced forwards into the pre-oral cavity, but such is not the case. The buccal shield is produced backwards ventrally, but the subneural gland lies in its primitive position in the collar, and is in no way produced into the pre-oral cavity. It is a backward ventral extension of the buccal shield which makes the subneural gland lie in front of the mouth,—not, as in *Balanoglossus*, a forward median extension of the subneural gland into the pre-oral cavity.

2. The Subneural Gland and Pharynx.—The subneural gland is an elongated caecal tube or prolongation of

the anterior wall of the pharynx. Its total length is usually about .14 mm., and its breadth about .02 mm. It usually has for about four fifths of its length a central lumen, which opens posteriorly into the pharynx, and terminates anteriorly in a variety of ways. Fig. 1 shows the subneural gland cut throughout nearly its whole length. The lumen usually, as in this case, contains a rod of glandular secretion of the nature of mucus. At its apex the gland is bent dorsally. Throughout the greater part of its extent its wall is composed of a simple glandular epithelium, but at its distal extremity the cells show a chordoid modification. The cells become vacuolated and reticular, producing the well-known chordoid structure of the "notochord" of *Balanoglossus*, *Actinotrocha*, and the *Vertebrata*. This is well seen in figs. 8 and 9. The extent of this chordoid modification varies immensely, and it is only the largest (oldest) individuals which show such a complete chordoid apex as in figs. 8 and 9. This specimen also shows a not uncommon feature in the complication of the central lumen. In the apex it forks out into two lateral canals as well as the median central canal (fig. 7)—a character also found in some *Enteropneusta*.

The relationships of the subneural gland to the pharynx have been already described elsewhere (10), and its connection with dorsal pharyngeal and peripharyngeal grooves has been demonstrated. In fig. 3 the commencement of the dorsal and peripharyngeal grooves is shown with their numerous unicellular glands. The commencement of the pleurochord is seen in fig. 5. It is important to notice the relationships of the subneural gland to the pre-oral and collar cavities. It is bounded laterally throughout its extent, except at the apex, by the walls of the two collar cavities, and ventrally by the wall of the pre-oral cavity. Above it the two collar walls form a median dorsal mesentery (fig. 2), and then diverge under the ectoderm to form the dorsal blood-sinus. At its distal end or apex the subneural gland reaches just beyond the collar walls, and plugs up the mouth of the heart, as described below (figs. 1 and 2).

3. The Pericardial Sac and Heart, Pre-oral Cavity, Pre-oral Canals, Glomerulus, Collar Cavities, and Blood-vascular System.—The pericardial sac lies anteriorly to the distal extremity of the subneural gland. In most specimens it is nearly square in cross-section, but may be compressed at its base as in fig. 6. Roughly its cross-section is about .05 mm., and its length about .08 mm. It appears to be a closed sac formed of very delicate endothelium; its posterior wall is invaginated to form the heart. This inner wall is thickened, and has numerous muscular fibres stretching across the cavity of the sac to its outer wall (figs. 6 and 2). It is doubtless contractile, and the shape of the pericardial sac varies greatly according to its state of contraction. On its ventral wall there is a fairly constant transverse groove (fig. 1). The sac lies in the blood-space or cavity between the walls of the pre-oral and collar cavities, and its walls do not differ except in their extreme delicacy from those of these cavities. In transverse sections it is seen that the pericardial sac is bent over the apex of the subneural gland dorsally and ventrally (figs. 7 and 8). Laterally it is bounded by the wall of the pre-oral cavity, which is thickened into an epithelial lining of the pre-oral canal. In fig. 6 both pre-oral canals are clearly seen, and the right pre-oral canal is cut throughout its length from the pore at the base of the ectodermal pit to the inner opening on the wall of the pericardial sac. The canal is lined by a delicate columnar epithelium, apparently ciliated. In this connection we may note the statement of Ehlers (2) that the "proboscis canals" of *Cephalodiscus* end in blind sacs. There can be no doubt whatever that McIntosh and Harmer were perfectly correct in stating that they open freely into the pre-oral cavity, though in a specimen examined as a transparent object the pre-oral canal might appear to terminate in the pericardial sac.

I have elsewhere (7) described the blood-vascular system of *Cephalodiscus*, and we have here to notice that, as indicated by Harmer (4), the organ I first took to be the

heart now proves to be a pericardial sac, containing the true heart¹ within it. The dorsal sinus can be seen running along immediately under the ectoderm and above the dorsal collar mesentery (figs. 1—4). Anteriorly it terminates against the posterior wall of the pericardial sac (which in a large number of specimens is ruptured). Here it is also joined on each side by a branchial vessel coming from the branchial plumes (fig. 7). Further, the anterior end of the dorsal sinus is continued into the cavity of the heart by paired lateral canals, the relationships of which are not easy to find nor to describe. If we could pull the apex of the subneural gland backwards from the mouth of the heart it is clear that the dorsal sinus would communicate directly with the heart. In the normal condition, however, this wide aperture of the heart is almost completely plugged up by the apex of the subneural gland. Dorsally and ventrally (figs. 7 and 8) this organ rests closely up against the pericardial wall, but laterally a small canal remains running downwards from dorsal sinus to heart (fig. 4). This canal is bounded posteriorly and laterally by the wall of the collar cavity, and anteriorly by the wall of the pre-oral canal (pre-oral cavity). It is doubtless through this paired canal that the blood finds its way from the dorsal sinus to the heart.

Below the subneural gland is a well-defined ventral sinus, which passes backwards to the level of the mouth and round it on either side. It is wide and large posteriorly, but passes forwards, getting narrower and narrower till it is lost in the glomerulus (figs. 7 and 8). Ventrally it is bounded by the wall of the pre-oral cavity, which also extends ventrally, laterally, and anteriorly to the pericardial sac. Various parts of this wall (pre-oral cavity) are thrown out into caecal prolongations into the cavity, with thickened protoplasmic walls. The cavities of these caeca are in direct communication with the blood-sinuses. They produce an appearance closely similar to that of the glomerulus or pericardial gland of *Balano-glossus*, with the exception that the walls are simple and

¹ The "pre-oral sac" of my previous work.

not of a definite cellular structure. There is usually a paired patch of this glomerular tissue on the antero-lateral surfaces of the pericardial sac (figs. 1 and 5) in close proximity to the internal apertures of the pre-oral canals. Further, the wall of the ventral sinus shows a similar structure (figs. 3, 4, and 7). In many cases the glomerular tissue of the ventral sinus is also paired, and the ventral sinus is then almost constricted into two paired sinuses.

There is little doubt that this glomerular tissue is homologous with the pericardial gland or glomerulus of *Balanoglossus*. Antero-dorsally to the pericardial sac we may notice a pre-oral sinus bringing blood back from the buccal shield (fig. 2) to the glomerulus.

We may now briefly run over the figures given here, noting the special points of each. Figs. 1 to 5 are selected from a series of very nearly sagittal orientation. In fig. 1 the subneural gland is cut almost throughout its length, its opening into the pharynx being more to the right. The right collar cavity is cut just at the apex of the gland, so that the sinus is rather more to the right anteriorly than posteriorly. The right glomerulus is also seen, whilst the cavity of the heart is spacious, although not at its largest (in the median line). The dorsal sinus is cut throughout its length, and two oral grooves may be recognised. In fig. 2 the collar mesentery is cut for some portion of its extent, and the glomerulus of the ventral sinus is coming into view; the left dorsal groove is also just appearing. In fig. 3 the pericardial sac is cut in the median line; the peculiar shape of the heart is noticeable. Further back the left collar cavity is alone seen, the dorsal sinus is restricted, and the subneural gland is interrupted. The left peripharyngeal and dorsal grooves are differentiated. In fig. 4 the heart is no longer visible, but the left canal from dorsal sinus into heart is seen. The posterior portion is still more to the left, showing the grooves as before. Fig. 5 is eight sections further to the left. In following the sinus one notices the left pre-oral canal becoming gradually more prominent, first laterally and then dorsally; the left glomerulus

appears, and the left collar cavity increases greatly in size. Posteriorly the first trace of the left pleurochord is seen, lying laterally to the dorsal groove. In this section the ganglion-cells are no longer seen, and the nerve is interrupted at the pre-oral canal.

In figs. 6 to 8 we have selected sections from a transverse series. The right side of the figures is slightly posterior to the left. Thus in fig. 6 the right pre-oral canal (on the left) is cut throughout its length, but the left only in part. In this section the pericardial sac is cut transversely, and the heart is seen in its greatest size. Fig. 7 is a few sections further back. Here the apex of the subneural gland is cut through, and shows three internal canals and a chordoid structure. Dorsally the pericardial sac is still cut, and below the ventral part is the glomerulus of the ventral vessel. On the right is seen the left branchial sinus leading out from the dorsal sinus, and a wider right branchial sinus opposite. The two horns of the ectodermal pit are also seen. Fig. 8 is still further back. The pericardial sac is no longer seen dorsally, but is still cut ventrally. The walls of the two collar cavities are approximately in the middle line, and behind the subneural gland will form a dorsal mesentery. The lateral nerves of the post-oral ring are seen in this section.

In fig. 9 the chief features here described are reproduced in a semi-diagrammatic median section of the entire animal. I have also shown the pharyngeal structure formerly described, i. e. the pleurochords, the dorsal and ventral grooves, and the oral grooves.

The facts above described must inevitably tend to bring *Cephalodiscus* into even closer union with *Balanoglossus* than heretofore. Not only is every organ in the central complex of the former to be directly compared to its homologue in the latter, but the latter has no organ in this region which does not occur in the former. The only essential difference is one which several years ago appeared to me to be of fundamental importance, but which must now be regarded as of secondary value. In *Balanoglossus* the pericardial

sac, glomerulus, and subneural gland protrude forwards into the pre-oral cavity, and hence are covered dorsally as well as ventrally; but in *Cephalodiscus* they protrude upwards between pre-oral cavity and collar cavities, and they are therefore dorsal and posterior to the former. In this way the pericardial sac lies in contact with the dorsal ectoderm, and the subneural gland is only separated therefrom by the dorsal sinus. This difference cannot be regarded as fundamental in view of the anatomical resemblance, and we have seen above that it is due to the forward protrusion into the pre-oral cavity of the subneural gland in *Balanoglossus*, whereas in *Cephalodiscus* it remains in the collar.

Of other points the homology of the subneural gland is a most important question. It appears desirable to adhere to this term, firstly, because it is unquestionably glandular in function; secondly, because it has precisely the same relationship to a system of dorsal and ventral grooves in the pharynx as is the case with the similarly-named organ in *Tunicata* (10); and thirdly, because its anatomical position is exactly under the main nerve-mass. These and other facts led me to doubt its homology with the "Eicheldarm" of *Balanoglossus*, but its relationships to pericardial sac and glomerulus and the chordoid structure of its apex appear to me to be conclusive in favour of accepting Harmer's original comparison. I would extend the appellation of subneural gland to the organ in *Balanoglossus*, for, as in so many other features, *Cephalodiscus* would appear to show us a more primitive condition of the organ than *Balanoglossus*. In making this comparison it appears to me to be questionable how far the subneural gland is at all comparable to the notochord of the Vertebrata. As indicated elsewhere (11, p. 412), a chordoid histological structure by itself cannot be regarded as an absolute criterion of homology, and the occurrence of chordoid organs of the same nature as, but not homologous with, the Vertebrate notochord is to be expected in these low chordates. The view of Willey that

Cephalodiscus is to be regarded as a degenerate ally of Balanoglossus has not much to commend itself; the consequent assumption that the former has lost numerous gill-slits perforating its anatomy in all directions, not to mention numerous other organs, has no justification in fact. We may with Lang (5) suppose that Cephalodiscus has undergone certain important modifications due to a semi-sedentary habit, but the assumption that its proximate ancestors had many pharyngeal clefts and gonads has nothing to recommend it but its necessity for Willey's theory. I would prefer to regard Cephalodiscus as the more primitive form, as its want of metameric segmentation and its primitive method of feeding would imply (9). On this basis the "Eichel-darm" of the Enteropneusta must be regarded as a glandular specialisation of the anterior end of the pharynx, to be termed the subneural gland, owing to its functions and structural relationships.

In Cephalodiscus its distal end often exhibits a commencing degeneration into chordoid tissue (which, by its development in Actinotrocha, is clearly an arrested form of glandular epithelium), whilst it is still functionally active as a gland. In Balanoglossus, with a specialised burrowing habit, the original function has been largely lost (though the "Eicheldarm" of Balanoglossus is unquestionably glandular), and the chordoid tissue with supporting function becomes still more in evidence. The organ to which the name of subneural gland was given in Actinotrocha occupies exactly the same position as in Cephalodiscus, but as it is only embryonic its walls would hardly be expected to be of a definitely glandular nature.

The pericardial sac of Cephalodiscus and its contained heart are so similar to the pericardium (Herzblase) and heart respectively of Balanoglossus, and so different from any structures found elsewhere, that the homology need not be insisted upon. In a similar manner the mutual relations of the ectodermal pit, the pre-oral canals, the pericardial sac, and the surrounding blood-sinuses speak for themselves.

Lastly, there can be little question that we have in the glomerulus a homologue of the proboscis gland of *Balanoglossus*. Each is a proliferation of the pre-oral cœlomic endothelium in the neighbourhood of the pericardial sac and pore canals, consisting of cœcal vascular processes.

It is evident that in the study of the budding processes (10) the origin of the pericardial sac must have been overlooked; but as we do not yet know how this organ arises in the demersal larva of *Balanoglossus*, nor even with certainty in *Tomaria*, this is not surprising. From certain indications it appears that in *Cephalodiscus* it is a portion of the pre-oral cavity constricted off from its posterior end, and therefore cœlomic in origin.

During the progress of my work on *Cephalodiscus* Cole (1) has published a short paper upon the bulbous terminations of the twelve branchial plumes. His results appear to indicate that the migration of oval lens-like bodies out of the epithelial cells to the exterior is to be regarded as a normal process, and that McIntosh's view (6) that these organs are masses of unicellular glands is correct. Assuming that the migration might be an abnormality, I had suggested that "it seems most reasonable to regard them tentatively as primitive eyes," a view I had already abandoned before the unexpected appearance of Cole's work. Cole further finds that the glandular bodies break up to form rhabdites, which I think quite probable, especially as I had already found and described indications of "one or more areas in the centre (of the bodies) staining more deeply than the rest (7)." I cannot agree with Cole's description of the epithelium in these terminal knobs as normally correct, as such a vacuolated swollen mass with little or no cuticle occurs commonly in other parts of the body, and seems to be an abnormality; the vacuolated condition of the bulbs is undoubtedly present, especially in older specimens. Cole denies the existence of a cuticle, of pigment, and of nerve-endings in the cells. In respect to the cuticle I am hardly prepared to inaugurate a discussion upon the line of distinction between a "peripheral deeply-staining

membrane and a cuticle. There is little doubt that the intracellular bodies under discussion arise in the young form in close contact with this limiting membrane, but it is possible that they do not actually arise from it. I am still of the opinion that fine brown pigment granules are scattered throughout the cells (McIntosh [6] previously remarked upon the "deep yellowish tint" of this region); and I still believe that the inner end of each cell "tapers to a fibre-like thread, which I believe to have in some cases traced into the main nerve of the plume" (7, p. 344). Indeed, it is rather difficult to understand otherwise in what region the very evident nerve down each plume terminates. None of these features are opposed to the "battery" function as suggested by Cole, though I have not as yet seen the rhabdites, which appear to require special staining. If their presence is corroborated it would form by no means the least interesting feature of *Cephalodiscus*. Cole, as a critic of the work of his predecessors, might perhaps make a somewhat more sharp distinction between a tentative suggestion and a definite statement of fact; but leaving this apart we may regard his work as confirming McIntosh's previous interpretation of the bulbous endings as masses of unicellular glands, the glandular secretion being extended to the exterior through the surface of the cells. Further, there is every reason to believe that, according to Cole, some at least of the glandular masses break up into rods.

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DESCRIPTION OF PLATES 32 & 33,

Illustrating Mr. A. T. Masterman’s paper “On the Diplochorda.”

FIGS. 1—5.—Selected sections (1, 3, 5, 7, 15) from a longitudinal sagittal series through *Cephalodiscus dodecalophus* (Zeiss, obj. 7, eyep. 1).

FIGS. 6—8.—Selected sections (1, 4, 6) from a transverse series through *Cephalodiscus dodecalophus* (Zeiss, obj. 7, eyep. 1).

FIG. 9.—A semi-diagrammatic right half of a polyp.



On Hypurgon Skeati, a New Genus and Species of Compound Ascidians.¹

By

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With Plates 34 and 35.

AMONG the marine sponges from the Malay Peninsula collected by Mr. R. Evans, of Oxford, at present Curator of the Government Museum in Georgetown, Demerara, and very kindly handed over to me by Dr. Harmer for description, there were included two specimens of the new genus of Synascida Didemnida, which I have endeavoured to describe below.

The locality named on the collector's label in the case of each of the two specimens is Pulau Bidang.

The association of the Tunicate with a sponge was merely fortuitous, and due solely to participation in the same surface of support.

The colony forms a thin sheet, little over 1 mm. in thickness, adherent to the substratum. The colour in spirit is a dirty yellowish brown.

The appearance of the colony when examined by reflected light under a low power of a binocular microscope is repre-

¹ I take the Greek *ὑπουργός*, and by lengthening the *ό* get *ὑπουργών*, meaning a place where things are made serviceable.

sented in Pl. 34, fig. 2. This external view shows at once the character to which the generic name alludes, namely, the presence in the test of numerous ovoid faecal pellets. These are seen through the transparent substance of the test, and now appear of an opaque cream-white colour. Clusters of calcareous asters (fig. 3) mark out the oral siphons, since they make a conspicuous snow-white patch around each siphonal aperture. These white spots are visible also with the naked eye.

The arrangement of the ascidiozooids is irregular. A large number of them share the same atrium, the atria being shallow but extensive cavities with but few and small siphons. The siphons are not visible in surface view, but in section it is seen that their lips are formed of transparent test-tissue destitute of spicules.

The bulk of the common test, which consists of actual tunicin, is small, its substance being excavated by numerous oval spaces, in which the faecal pellets lie. To reach this position the pellets must, after being ejected into the atrium, sink through the excessively thin epithelial wall of that cavity. The cellular elements in the test are of the usual types; bladder-cells are specially abundant near both upper and under surfaces, and round the oral siphons. Spicules occur in small numbers, chiefly aggregated round the oral siphons and in the neighbourhood of the branchial sac. They may be isolated or packed in dense clusters (Pl. 34, fig. 3). Finally the renal vesicles, described presently, are to be reckoned among the structures included in the test. The ascidiozooids, as is common among *Didemnida*, have a sharp constriction between the branchial region of the body and the abdomen.

The number of lobes round the oral siphons varies from four to six. The tentacles are twenty-four in number; twelve long ones alternate with twelve short. The branchial sac has four rows of five stigmata on each side. Connectives (Hancock; trabeculae, Yves Delage) are absent. The dorsal languets are long and median in position. The sub-

neural gland has a simple opening with a swollen lower lip (fig. 4, *d. t.*).

Through the narrow aperture of communication between the two regions of the body the œsophagus descends to open into the stomach, while the intestine passes upwards into the rectum, which lies above the constriction, so that the anal opening is close to the base of the branchial chamber.

The walls of the stomach are raised up round the termination of the œsophagus; or, in other words, the œsophagus has its opening deep in the cavity of the stomach; the terminal part of the œsophagus is richly ciliated. The intestine of a young bud is frequently found attached at both ends to the œsophagus, to which it owes its origin. When this is the case the thoracic portion of the same bud is to be seen lying in the test at the opposite side of the œsophagus. The budding is thus of the type known as pyloric (Giard), and found among Didemnidæ in the tribe Didemminæ (Y. Delage).

The walls of the stomach are smooth; seen en face from the outside they show a beautiful reticulum formed of the more deeply staining protoplasm which surrounds and connects the nuclei of the cells of the gastric epithelium.

The intestine as it leaves the stomach is richly ciliated; in passing thence to the anus its walls become continually thinner, the walls of the rectum being almost membranous. The anus has thickened lips. The alimentary canal is bathed by blood-sinuses along its whole course.

The heart in its pericardium runs more or less vertically between the upper and lower walls of the abdominal cavity. Its lower end abuts against and sends a large vessel into a prominence of the test, the sides of which are covered by a patch of specially large cells of the mantle which form the glandular part of the renal organ (*r. gl.*, figs. 5 and 7). The excreta of these glandular cells appear to be picked up by wandering cells—presumably corpuscles of the blood contained in neighbouring vessels or sinuses. These cells would then migrate into the test, carrying their burden with them.

Large numbers of vesicular cells containing concretions are to be found embedded on each of the above-mentioned prominences of the test, while in older kidneys there may be a relatively enormous rounded mass of such vesicles more deeply situated in the test substance (fig. 7, *k*). Some such masses may be found in the basal layers of the test at a distance from the abdominal cavity of any zooid; these have evidently been left behind, the zooid to which they belonged having shifted upwards as the floor of the cloacal cavity was raised by the continual addition of fresh pellets.

Thus the excretory organs of *Hypurgon* agree with the simple type of excretory organ found in *Botryllus*, in that the urinary concretions are stored in the cavities of single vesicular cells; but apart from this particular they are of a type unlike any yet described (Dahlgrün, 'Archiv für mikr. Anat.,' vol. lviii, 1901) among Tunicates, and are far less simple than any known in other Synascida.

The reproductive organs lie in shallow depressions of the wall of the abdominal cavity (fig. 9). The testis is oval, and the vas deferens makes four or five turns of a spiral around it. The ovary has membranous walls, and contains a string of eggs of successive ages. I have not seen an oviduct.

Any mature ova that I have seen have sunk deep into the test, and so have come to lie in a great recess of the abdominal cavity (fig. 10), communicating with it by a narrow aperture. The material contains but one larva, which was developing in a completely closed cavity in the test (fig. 11). This may or may not be the normal course taken by the developing eggs. Eggs are not to be seen being sheltered by any other part of the organism than the test, though eggs of all ages were found in the ovaries.

The faecal pellets, which contribute so largely to the formation of the test, show a very remarkable degree of coherence. If a piece of the colony be boiled in sulphuric acid, the residue consists of faecal pellets which retain their form perfectly, and continue to do so even if the boiling be much prolonged. Even thin sections of pellets, isolated by

boiling microtome sections of the colony in sulphuric acid, may still be mounted whole after this treatment. Boiling in aqua regia and boiling in fuming nitric acid are equally ineffectual in disintegrating the pellets; when these latter reagents are used the test naturally forms part of the residue, since they are not capable of dissolving tunicin.

When isolated by means of sulphuric acid the pellets have a black colour, due to the action of the acid on the organic matter contained in them. These blackened pellets may next be washed and calcined, and though raised repeatedly to cherry heat they still remain intact, and are now opaque white when examined by reflected light. Mounted in oil, or passed through oil into balsam, they become transparent. Calcined pellets dissolve completely in hydrofluoric acid. Prolonged boiling in a strong solution (nearly saturated) of caustic soda resulted in the dissolution of calcined pellets.

It seems, then, that the strong coherence of the pellets must be due either (1) solely to cohesion and adhesion between the foreign particles contained in them, or (2) to a deposition of silica between these particles. The siliceous nature of the greater part of this foreign matter makes it impossible to determine between these two alternatives. It naturally suggests itself that this property of coherence of the pellets is an adaptation to enable the animal to utilise waste organic matter with impunity. But it must be mentioned that the pellets are porous, taking stains readily both before the treatment described above, and also at every stage during it.

It is curious that the pellets are also highly fragile; they crumble at once under pressure of the cover-slip.

Melicerta tubes were boiled in acid for comparison: the form of the component pellets was lost almost immediately—as soon as the cementing substance between neighbouring pellets disappeared.

A parasitic crustacean was found in one ascidiozoid, occupying a large part of its branchial chamber. The body of the parasite is a mere sac filled with ova in an advanced state of segmentation. There appear to be six pairs of

appendages belonging to the anterior region of the body, besides one foremost pair which serves as an organ of attachment, and is inserted into the tissues of the host.

The systematic position and diagnosis of the genus may be stated as follows:—Synascida Didemnida Didemnina, (Y. Delage). Colony thin; ascidiozoids with four rows of branchial slits and twenty-four tentacles; vas deferens spirally coiled round the testis; faecal pellets included in the test, in which organ the renal vesicles are likewise contained.

In conclusion, it gives me much pleasure to take this opportunity of expressing my thanks to Mr. Graham Kerr for kind help and advice.

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EXPLANATION OF PLATES 34 & 35,

Illustrating Igerna B. J. Sollas’s paper “On Hypurgon Skeati, a New Genus and Species of Compound Ascidians.”

as. Calcareous spicule. *at. s.* Atrial siphon. *bd. c.* Blood-corpuscle. *bl. c.* Vesicular cell of test. *bl. s.* Blood-sinus. *d. l.* Dorsal languet. *d. t.* Dorsal tubercle. *end.* Endostyle. *f.* Fusiform cell. *g.* Nerve ganglion. *h.* Heart. *int.* Intestine. *l.* Larva. *n. tc.* Notochord. *œ.* Œsophagus. *ov.* Ova. *p.* Faecal pellet. *p. c.* Pericardium. *rect.* Rectum. *r.* Renal organ. *r. gl.* Glandular cells of renal organ. *r. c.* Renal concretion. *st.* Stomach. *t.* Testis. *v. app.* Vasc. appendage. *v. d.* Vas deferens.

PLATE 34.

FIG. 1.—A piece of a colony of *Hypurgon Skeati*, slightly larger than natural size.

FIG. 2.—A portion of the surface of a colony seen under a binocular microscope. $\times 75$.

FIG. 3.—Calcareous spicules from the test of *Hypurgon Skeati*. *a* and *b* from one colony; *c*, *e*, and *f* from a second. *f*, a cluster of spicules.

FIG. 4.—A vertical section through a part of a colony of *Hypurgon Skeati*, showing the branchial sac and parts of the abdominal cavity of one zooid (slightly reconstructed from neighbouring sections). $\times 80$.

FIG. 5.—Vertical section of an abdominal cavity.

FIG. 6.—Diagrammatic reconstruction of a slice of a colony of *Hypurgon Skeati*, showing one zooid from the left side and one from the dorsal surface. Drawn as though it were transparent.

PLATE 35.

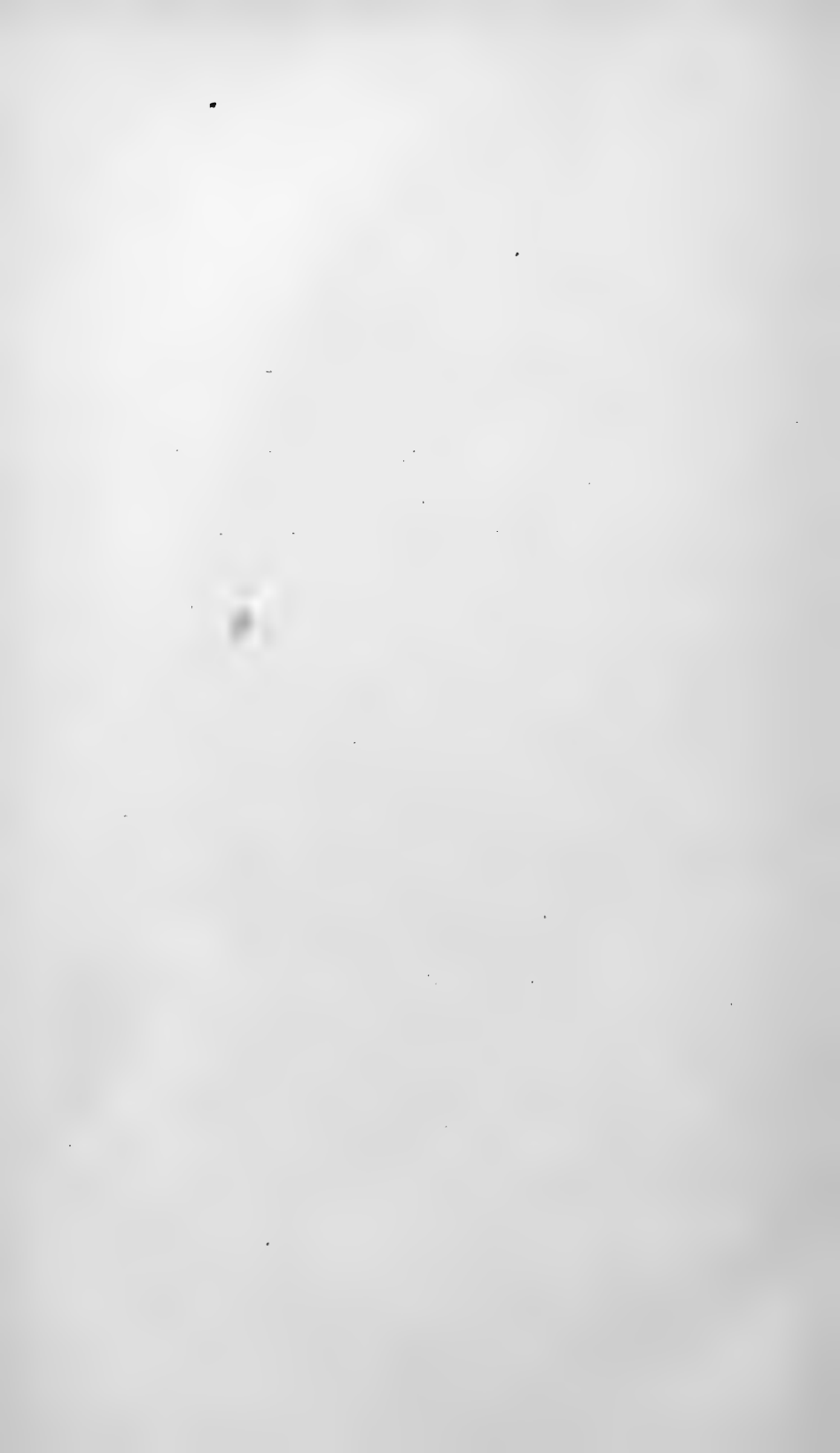
FIG. 7.—Section of a renal organ of *Hypurgon Skeati* which has been functioning long enough to form the considerable accumulation of concretions *k*.

FIG. 8.—Portion of the test of *H. Skeati* containing renal vesicles, more highly magnified.

FIG. 9.—Section of an abdominal cavity of zooid of *H. Skeati*, to show reproductive organs.

FIG. 10.—Section of a large ovum of *H. Skeati* in a recess of the abdominal cavity.

FIG. 11.—Section of a tailed larva of *H. Skeati* developing in a closed cavity in the test.



**The Anatomy of *Arenicola assimilis*, Ehlers,
and of a New Variety of the Species, with
some Observations on the Post-larval Stages.**

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With Plates 36 and 37.

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I. Introduction.

IN response to my inquiry regarding the occurrence of *Arenicola* on the shores of New Zealand, Professor Benham kindly sent to me three specimens of this worm from Otago Harbour, and one from the Macquarie Islands.

The specimens were caudate Arenicolidæ resembling *A. marina*, Linn., and *A. claparedii*, Levinsen, in external form. A rapid examination of the grosser anatomical features of one of the Otago specimens seemed to point to its

close affinity with the latter species, for it was at once seen that the New Zealand specimen possessed multiple œsophageal glands and that there were no pouches on the first diaphragm—two features known only in, and considered to be almost diagnostic of, *A. claparedii*. At first, also, only five pairs (the number occurring in *A. claparedii*) of nephridia were seen in the Otago specimen, but finally a much reduced pair was found in the segment anterior to the one bearing the first fully developed nephridia. In the other three specimens sent by Professor Benham there are six pairs of fully developed nephridia, which is evidently the normal condition. The lateral lobes of the prostomium of these specimens were found to be more feebly developed than those of *A. claparedii*. There were therefore two points in which the southern specimens agreed with Levinsen's species, viz. the presence of multiple œsophageal glands and the absence of diaphragmatic pouches; and two features in which they differed, viz. the form of the lateral lobes of the prostomium and the number of nephridia.

On sectioning the anterior end of one of the Otago specimens a pair of large otocysts was found, each opening to the exterior by a narrow tube. The presence of these well-developed organs, in conjunction with the important differences above mentioned, finally settled that the New Zealand specimens do not belong to the species *A. claparedii*, in which the absence of otocysts is so characteristic and remarkable a feature.

These specimens agree with *A. marina* in the number, position, and character of their gills, in the number of their nephridia, and in the general anatomy of their otocysts; but the southern specimens are clearly distinguished from *A. marina* by their multiple œsophageal glands, by the absence in the former of diaphragmatic pouches, and by other less obvious features.

The only other known species to which the New Zealand specimens show any close similarity is *A. assimilis*, Ehlers (1897, pp. 103, 104), of which only the external

characters are briefly described. Ehlers states that this species closely resembles *A. marina* in general external characters, but that in *A. assimilis* there are twenty chætigerous segments and as a rule there are thirteen pairs of gills, the first being situated on the eighth chætigerous annulus, but occasionally only twelve pairs of gills are present. Ehlers also finds that, compared with *A. marina*, the median lobe of the prostomium of *A. assimilis* is proportionately smaller than the lateral ones, and the notopodial setæ somewhat more feebly feathered.

The New Zealand specimens differ from Ehlers' species in the number of chætigerous segments and in the position of the first gill, but unfortunately Ehlers does not mention the nephridia or nephridiopores, the œsophageal glands or the otocysts,—important diagnostic characters concerning which information was essential before the affinity of the New Zealand specimens with *A. assimilis* could be either accepted or rejected. My thanks are due to Dr. Michaelsen, of the Hamburg Museum, who collected the specimens examined by Ehlers, for kindly sending to me two complete examples of *A. assimilis* from Uschuaia, in Tierra del Fuego, and an incomplete specimen from Punta Arenas, in the Straits of Magellan.

As Ehlers has given only a brief account even of the external characters of his species,¹ I propose to describe the principal features of the three specimens given to me by Dr. Michaelsen before proceeding to consider the New Zealand specimens in detail.

¹ Since writing the above I have received, through the kindness of Professor Ehlers (1901), a copy of his recently published monograph, 'Die Polychæten des magellanischen und chilenischen Strandes,' in which he describes (pp. 177, 178) the structure of the otocysts of *A. assimilis*, and also states that the alimentary canal, the vascular system, and the nephridia of this species agree, so far as he can ascertain, with those of *A. marina*. This agreement is, however, not quite so close as Ehlers' statement would lead one to expect, since, for example, the œsophageal glands in the latter species are a single pair, while in *A. assimilis* there are several pairs. These points are further discussed below.

II. *Arenicola assimilis*, Ehlers.

The two complete specimens found at low-tide mark at Uschuaia are 105 mm. and 120 mm. long respectively. The specimen from Punta Arenas would have been about 120 mm. long if complete. Their colour in spirit is a fairly uniform yellowish brown, but the tail of one specimen is of a somewhat darker colour. The body is slightly swollen in the anterior region. The animal closely agrees in form with specimens of *A. marina* of the same size.

External Characters.—The prostomium (see fig. 20) is moderately developed; the two lateral lobes are in the form of a **V**, the arms of which embrace the median lobe.¹ The prostomium resembles that of *A. marina* and *A. cristata*, except that the median lobe is proportionately smaller in Ehlers' species. The nuchal organ is similar in its structure and relations to that of *A. marina*. The metastomial grooves indicating the track of the œsophageal connectives are well marked.

There are twenty chætigerous segments, the last thirteen of which are branchiferous; the first gill is thus on the eighth chætigerous annulus. In this character *A. assimilis* differs from all other caudate Arenicolidæ, in which the first gill (except in those abnormal cases in which the first true gill is missing) is on the seventh chætigerous annulus. Of the three specimens in my possession only one has thirteen pairs of fully developed gills; in another the first right gill is very minute; while in the third specimen the first left gill is small, and the last gill on each side is considerably smaller than the preceding one. Ehlers (1897, p. 104) records specimens with only twelve pairs of gills.

The gills of the specimens from Uschuaia are dense bushy structures resembling the dendritic type of gill found in

¹ There is a rough sketch ("d'après Ehlers") of the prostomium of *A. assimilis* in a memoir by Fauvel (1899, p. 178), which, however, does not give an accurate impression of its form, as the two lateral lobes are represented as separate, whereas they are actually united posteriorly to form the V-shaped structure described above.

littoral specimens of *A. marina*. There are in most of them about eight main stems, each 2.5 mm. to 3 mm. long, bearing five or six dichotomously subdivided branches on each side. The gills of the specimen from Punta Arenas are larger and of a somewhat more regular form. The eight or nine stout main stems are about 4 mm. long, and are regularly arranged in radiating fashion; each bears six or seven pairs of branches which divide dichotomously. Although at first sight this gill seems to approach to the pinnate type, the lateral branches are neither so numerous nor so regularly arranged as in pinnate gills, and the gill may be regarded as merely a well-developed example of the dendritic type.

The skin is subdivided into annuli. Between the prostomium and the first chætigerous annulus there are five rings (see fig. 20). The first four of these represent the region found in other species of *Arenicola* which has been shown to be composed of the peristomium (here represented by the first two of these rings) fused with the first body-segment of the post-larva, the setæ in which disappear very early (see figs. 19, 20). The fifth ring is the first annulus of the first chætigerous segment, this segment being composed of three annuli, viz. a chætigerous one and the annulus preceding and following it. The second and third chætigerous segments also consist of three annuli, the middle one bearing the setæ. The fourth and succeeding segments up to the end of the branchial region are composed of five annuli, the fourth of which is chætigerous. The region between any two chætigerous annuli behind the third is therefore subdivided into four rings.

The epidermis of the tail is raised into numerous papillæ. The segmentation of this region is only feebly marked, but it is indicated, especially in the anterior portion of the tail, by the presence of somewhat larger annuli placed at regular intervals, upon which the epidermal papillæ are distinctly larger than those on the intervening annuli. Each of these larger rings is followed by a slight constriction, denoting the presence internally of a septum, best seen in those parts of

the tail which were stretched at the moment of death. The space between two of the larger annuli is subdivided at the anterior end of the tail into two or three rings, but further back into from four to ten. These smaller annuli also bear epidermal papillæ, but in the anterior tail region they are distinctly smaller than the papillæ found on the larger annuli. Proceeding backwards along the tail, the difference in the size of the annuli and of the papillæ they bear may be clearly recognised until the middle of the tail has been passed; then the papillæ become almost equal in size, and near the anus it is impossible to distinguish any difference between those of the various annuli. There are about twenty-eight segments in the tail of each of the complete specimens.

Setæ.—The capillary setæ (figs. 1, 1A) of the notopodium are very similar to those of *A. marina*. They attain a length of 4.3 mm., and on their distal fourth bear small pointed processes, which, as Ehlers (1897, p. 104) remarked, are not so well developed as those of *A. marina*. The processes are usually present on both sides of the seta; they are moderately obvious on one side, but on the other they are very minute, and are borne on the edge of a thin border or lamina. This lamina, which seldom exceeds 6μ in width, extends along the seta for a distance of about one third its length. In some of the setæ the lamina is not denticulate at its margin, and in others is only very faintly so; but it is crossed by fine oblique lines, the intervals between which correspond roughly to the size of the teeth on the dentigerous laminae. From an examination of the setæ of *A. assimilis* and *A. marina*, it seems probable that the lamina at first possesses an entire margin, but later this tends to break up from the edge inwards, thus giving rise to the minute teeth which are usually seen on full-grown setæ. This explanation would account for the fact that in some setæ the margin of the lamina is entire, while in others it bears either exceedingly minute denticulations or the more obvious teeth shown in fig. 1A. These three conditions are occasionally seen at different points along the border of a single seta.

Setæ similar to those above described are present in *A. marina*, and in some examples the lamina is very well marked, e. g. in a specimen of the Laminarian variety the thin border extends for nearly a millimetre along the seta, and attains a width of 20 μ . Similar setæ are present in *A. cristata*, but in *A. claparedii*¹ the lamina is not so well developed, being short and narrow. In *A. ecaudata* and *A. grubii* the lamina is also very narrow, seldom exceeding about 3 μ in width.

The neuropodia of *A. assimilis* are easily seen, even in the first segment. They are especially well developed in the branchial region, where each resembles a pair of closely applied tumid lips, between which is the row of crotchets. These (fig. 9) are often curved, and are 0.6 mm. to 0.7 mm. in length, being considerably longer than chætæ from specimens of *A. marina* of the same size. (The longest crotchets found in a specimen of *A. marina* 125 mm. long are only 0.47 mm. in length.) The rostrum is short and blunt, even in unworn chætæ. There is a small subrostral enlargement, and about six to nine teeth are present just behind the rostrum.

Musculature.—The musculature calls for little comment; it is similar to that of *A. marina*, except that the oblique muscles are present along the whole animal from the first diaphragm to the end of the tail. They are exceedingly thin bands, somewhat broader in the posterior part of the gill region, but even here seldom exceeding 0.5 mm. in width, and as a rule they are only 0.2 mm to 0.3 mm. wide. There is a dorsal mesentery in the first and second chætigerous segments supporting the dorsal blood-vessel. The three diaphragms are, as usual, situated at the anterior ends of the first, third, and fourth chætigerous segments. There are no pouches on the first diaphragm. This condition was considered to be so marked a feature of *A. claparedii* that it was given as one of the diagnostic characters of this species (Gamble and Ashworth, 1900, pp. 533, 541), since all other Arenicolidæ whose anatomy is fully investigated possess

¹ See Gamble and Ashworth, 1900, pl. xxiii, fig. 23.

diaphragmatic pouches, and in some species they attain a large size, e.g. in *A. cristata* they may reach a length of 12 mm. Many of the blood-vessels which cross the coelom obliquely to the nephridia and gills are provided with a very obvious connective-tissue strand or band, which gradually increases in size in the posterior segments of the gill region, forming in the last two or three segments of this part of the body an almost complete septum supporting the afferent and efferent branchial vessels. There are well-developed caudal septa.

Alimentary Canal.—The most striking feature of the internal anatomy of *A. assimilis* is the presence of multiple oesophageal pouches. These are placed on the sides of the oesophagus, just behind the third diaphragm. There are in each of two specimens six, and in another eight, pouches on each side. The anterior pair is long—12 mm. in one specimen, 17 mm. in another,—and each of them is usually swollen at or near its anterior free end, having a club-shaped appearance. Their abundant blood-supply is evidenced by the network seen in their walls. In the contracted condition these anterior pouches are digitiform structures with a somewhat moniliform appearance. The smaller posterior glands are from 1 mm. to 4 mm. in length, and are pear-shaped or oval sacs with rather thicker walls. As in other *Arenicolidae*, the cavity of each pouch is partially subdivided by numerous septa produced by infolding of the wall; each septum, therefore, is composed of two lamellæ of glandular epithelium, between which is a cavity filled with blood. The partitions are very obvious in the smaller pouches, and in the larger pouches when in a contracted condition; but when these are fully distended the septa become mere ridges on the inner wall of the pouch. In the presence of multiple oesophageal pouches *A. assimilis* conforms to another of the features hitherto considered to be peculiar to *A. clapedii*, as in all other species of *Arenicola* in which the pouches are known there is only a single pair.¹ In other

¹ Ehlers (1901, p. 177) states that the gut of *A. assimilis* agrees with

respects the alimentary canal resembles that of *A. marina*. The ventral groove, which is well seen in the intestine, may be traced forwards into the stomach to about the level of the eighth or seventh seta.

Vascular System.—The vascular system closely agrees with that of *A. marina* (see Gamble and Ashworth, 1898, pl. ii), except in the fifth and seventh chaetigerous segments. In each of these there is only one pair of vessels, afferent branches of the ventral vessel, passing to the nephridia. The first pair of efferent branchial vessels is situated in the eighth segment; this and the four succeeding pairs open into the subintestinal vessels, while the last eight gills, i. e. those of the thirteenth to twentieth segments, return blood to the dorsal vessel. The body-wall is well supplied with blood-vessels, especially in the anterior region; in sections of the peristomium and first chaetigerous segment there are numerous vessels lying either in the connective tissue or in small coelomic canals (see below) just beneath the epidermis (fig. 22); in sections of some of the posterior segments the vessels are not so abundant.

The heart is of moderate size, and has the usual relations. There is a cardiac body formed by ingrowths, chiefly of the posterior wall of the heart, and this is well developed in one of the specimens 120 mm. long.

Coelom.—The coelom is spacious, as in *A. marina*. A remarkable feature noticed at once in sections (fig. 22) of the anterior end of *A. assimilis* is the large number of coelomic spaces in the body-wall and between the muscles. In this portion of the animal there are exceedingly numerous coelomic canals lying in the subepidermal tissue of the body-wall, penetrating into the muscle-bands, especially of the buccal musculature, insinuating themselves between the brain-lobes and between the brain and the prostomial epithelium, and often accompanying the blood-vessels which supply the body-

that of *A. marina*, but the presence of multiple oesophageal glands in the former while there is only a single pair in the latter species is a point of difference of considerable systematic importance.

wall. In each of these canals the thin lining of coelomic epithelium may be easily recognised, and coelomic corpuscles may also be found in many of them. Similar canals are present in *A. marina* (Gamble and Ashworth, 1898, p. 28), in *A. grubii*, and to a less extent in the other species; but the development of these outgrowths of the coelom reaches its maximum in *A. assimilis*. They probably act as nutritive, and possibly also as excretory and respiratory channels. There are very few coelomic canals in the posterior part of the animal. The coelomic fluid and corpuscles resemble, as far as can be ascertained in preserved specimens, those of *A. marina*.

Nephridia.—There are six pairs of nephridia, the external openings of which are slightly posterior to the dorsal ends of the fourth to the ninth neuropodia. The funnels of the first pair of nephridia lie on the anterior face of the third diaphragm. This condition is found again only in *A. marina*; the first pair of nephridia of other species corresponds in position to the second pair of *A. assimilis* and *A. marina*. The dorsal lip of each nephrostome (fig. 17) bears about twelve to fourteen spatulate or triangular ciliated processes attached by their narrower ends. These are subdivided distally, some of the larger ones into five or six. The edge of the ventral lip of the nephrostome is thrown into folds or frills, so that although it agrees in general shape with the ventral lip of the nephrostome of the *marina* section of the genus, the ventral nephrostomial lip of *A. assimilis* is quite distinguishable by this peculiar character. This frilling is probably not due to contraction on killing, as it is not seen in specimens of *A. marina*, *A. claparedii*, and *A. cristata* which have been killed in a similar manner.

The nephrostome of the first nephridium lies on the anterior face of the third diaphragm, and is directed anteriorly. It is smaller than any of the other nephrostomes, its dorsal lip bears only eight to eleven processes, and the frilling of the edge of the ventral lip is not so well marked.

The first nephridium is usually distinctly smaller than any of the others, a condition frequently noticed in *A. marina*. Although this nephridium possesses a gonidial vessel, no gonad is developed upon it.

Gonads.—The gonads are, as in other species, associated with the nephridia, and are present on all except the first pair. Each gonad is a club-shaped mass of cells about 1.5 mm. long (fig. 17), formed by proliferation of the cells covering the gonidial vessel (Gamble and Ashworth, 1900, p. 521) immediately behind the nephrostome. The formation of ova and spermatozoa follows the same course as in *A. marina*. The ova present in the coelomic fluid of the specimen from Punta Arenas are apparently mature,¹ and have a distinct but thin vitelline membrane (3μ thick). They are not spherical, but somewhat discoidal. The face of the disc is usually oval, and measures 0.19 to 0.20 mm. by 0.15 to 0.16 mm. in diameter. The thickness of the egg is about 0.075 mm. Measurements of a considerable number of well-preserved unshrunk eggs from the coelomic fluid show that the three axes above named are fairly constant in proportion. It will be convenient to correct here a statement in the memoir by Dr. Gamble and myself (1900, p. 527) in which the ova of *A. marina*, *A. claparedii*, and *A. cristata* are described as spherical. This is a mistake, as the ova of all these species are flattened in one plane, like those of *A. assimilis* described above. In *A. marina*² the face of the egg is either circular (usually) and about 0.14 to 0.15 mm. in diameter, or it is oval, with diameters of 0.16 and 0.12 to 0.14 mm., and the third axis of the egg is from 0.08 to 0.09 mm. in length. The ova of *A. claparedii* are usually only slightly oval, the two diameters of the face of the egg being about 0.16 mm. and 0.14 to 0.16 mm. respectively, and the

¹ This specimen was taken in September, 1892.

² The following description and measurements may replace those given on p. 527 in the memoir cited above; they have been drawn either from living or well-preserved ova, most of which have come into my hands since the completion of that memoir.

thickness of the egg 0.07 mm. In *A. cristata* the three axes of ova removed from a ripe female in Naples measure 0.155 mm., 0.145 mm., and 0.07 mm. respectively.¹ The ova of *A. grubii* and *A. ecaudata* are not compressed in this way, or only very slightly so. They are usually ovoid, and ripe ova of the former species are 0.17 mm. long and 0.15 mm. broad and thick. The largest ova of *A. ecaudata* which I have seen have slightly smaller dimensions, but they are probably not quite mature. The ova of *A. grubii* and *A. ecaudata* are distinguished by their stout vitelline membrane, which is 5 to 6 μ thick; while in *A. clapedii*, *A. cristata*, and *A. assimilis* it is 2 to 3 μ , and in *A. marina* only slightly over 1 μ in thickness.

Brain.—The brain of *A. assimilis* conforms to the general plan seen in the *marina* section of the genus. It consists of a pair of anterior lobes placed well forward in the prostomium, a pair of posterior lobes which lie below the nuchal organ, and an intermediate region which connects the anterior and posterior lobes. The anterior lobes are short but very broad; in fact, this is by far the broadest part of the brain; behind these lobes the brain gradually tapers. The brain may be roughly compared in shape to two slightly flattened pears lying side by side with their narrower faces adjacent and fused along the middle third of their length. The broad forwardly directed ends of the pears represent the anterior cerebral lobes, while the tapering ends represent the posterior lobes, which are continuous with two nerve-tracts lying below the epithelium of the nuchal organ. The anterior brain-lobes are separated in front by a coelomic space. Each gives off anteriorly and dorsally a series of nerves to the epithelium of the prostomium. The anterior part of these lobes consists almost entirely of small cells situated in clusters and separated from one another by fibrous tracts and by neuroglial tissue. Further back the delicate neuropile which forms the core of these anterior lobes is well seen,

¹ See also Child (1900), p. 592, for further observations on the ova of *A. cristata*.

surrounded by clusters of nerve-cells and neuroglial tissue. Bundles of nerve-fibrils may be traced from the bases of the prostomial epithelial cells into the neuropile (see fig. 23). Larger unipolar ganglion-cells are found immediately outside the neuropile, and particularly on the side nearest the middle line. The œsophageal connectives arise from the anterior lobes at the point where the neuropile reaches its greatest development (fig. 22). The eyes are found on the dorsal side of this part of the brain. There are four or five on each of the anterior lobes. A little further back large pyriform ganglion-cells become more numerous, and especially on the inner side of the two anterior lobes just before they unite in the middle line and for some distance after their union (see fig. 23). Passing backwards along this region, it is seen that the ganglion-cells become more restricted to the dorsal and lateral faces of the brain, the middle and ventral parts being composed largely of neuropile, in which also neuroglial cells and fibrillæ may be recognised. The ganglion-cells of this region are more intimately associated with the median part of the prostomium.

The posterior brain-lobes are small and tapering, and gradually merge into two nerve-tracts which lie on the inner side of the nuchal organ just below the sensory epithelium. The brain of the specimen 120 mm. long is 0.65 mm. in length, 1 mm. broad across the anterior lobes, and about 0.4 mm. deep in this region. It is most nearly like the brain of *A. marina*, but is wider anteriorly. (The brain of a specimen of *A. marina* 120 mm. long is about 0.7 mm. wide.)

Æsophageal Connectives.—The œsophageal connectives arise from the lateral region of the broadest part of the anterior cerebral lobes, i. e. just in front of their point of union. Each is a stout fibrous cord with numerous cells on its outer face, which, in the first part of its course, lies about a millimetre below the epidermis, and is slung up in a muscle sheet, which is attached to the subepidermal musculature by numerous muscle-strands (fig. 22). It is only in the ventro-lateral region that the connectives approach the

body-wall and finally come to lie upon the layer of circular muscles. The connectives, the course of which is indicated externally by the metastomial grooves, unite in the hinder portion of the third annulus (cf. fig. 19). Each gives off numerous branches to the epidermis of the region through which it passes; in fact, the nerve-supply to the skin and following segment is enormous—nerves pass into the raised areas upon the skin and repeatedly branch, their terminations lying in close contact with the bases of the epidermal cells (fig. 22). In addition to one or two nerves derived from each connective, the skin of the region immediately below the prostomium receives two moderately stout nerves which arise from the ventral portion of each anterior cerebral lobe close to the point of origin of the connective. These nerves run on to the roof and sides of the mouth, and their branches may be traced a considerable distance along the pharynx. They are apparently more numerous on the dorsal than on the ventral region of the pharynx. In many sections the nerves may be seen sending branches along the axes of the buccal papillæ (fig. 22). The connective gives off a very short but stout nerve to the otocyst. Ganglion-cells are present in moderate number around the point of origin of this nerve. The nerve comes into contact with the otocyst at the point where the tube leads off to the exterior, and is intimately related to both structures. It provides the otocyst with a sheath of nervous elements, which lies just below the sensory epithelium, and also sends a small nervous sheath along the tube.

Nerve-cord.—The nerve-cord is situated within the layer of circular muscles. The right and left fibrous portions are separated by a median vertical sheet of neuroglia. The ganglion-cells are distributed along the whole length of the cord, and are not aggregated into ganglia. They are numerous, unipolar, pyriform, usually quite small cells with deeply staining nuclei, and most of them are situated in the ventro-lateral regions of the cord; but some few larger ones measuring from $15\ \mu$ to $30\ \mu$ in length, and having vesicular

nuclei, are found rather nearer the middle line in many of the sections examined. The process of each cell is directed dorsally into the lateral portion of the fibrous mass. The spinal nerves arise in the same manner and position as in other Arenicolidæ (Gamble and Ashworth, 1900, pp. 482, 483). Giant-fibres to the number of two or three are seen in sections of the branchial region and tail. The giant-cells are regularly arranged, being situated close to the posterior border of each segment. In eight of the nine segments examined there is only one giant-cell per segment, but in the other segment two cells are present near together. The giant-cells are placed in the extreme lateral regions of the cord, and in the first piece of cord which was sectioned they are situated alternately on the right and left sides, i. e. in the seventeenth to twentieth chætigerous segments, and in the first tail segment of this specimen they are situated respectively R, L, R, L, R (see fig. 12). In sections of the same specimen taken further forwards (tenth to thirteenth segments) this curiously regular arrangement was not found, the cells present in these segments being situated respectively L, L, R R (two cells are present in this somite), R. The average size of the cells is 0.065 mm. long and 0.05 mm. broad and deep. Each cell is pyriform, surrounded by a fibrillated sheath, and sends out usually only one process, which passes at once into the fibrous portion of the cord towards the lateral giant-fibre. The protoplasm of one cell, however, is drawn out dorsally into five processes, one of which is much thicker than the others, and may be easily traced into the mid-dorsal region of the fibrous part of the cord. The slender processes are traceable only a very short distance, being lost either between the small ganglion-cells or immediately on entering the fibrous part of the cord. There is in most of the giant-cells a more deeply staining area in the protoplasm close to the nucleus, due to the presence of chromophilous granules. This probably corresponds to the similar but better marked centrosphere seen in *A. grubii* (Gamble and Ashworth, 1900, pp. 487, 488, and fig. 76).

Sense Organs.—The eyes, of which there are four or five on each side of the prostomium, are similar to those of other Arenicolidae (Gamble and Ashworth, 1900, pp. 506, 507). They are situated either among the small nerve-cells on the dorsal surface of the anterior cerebral lobes just in front of their point of union, or in the epidermis immediately dorsal to this region. Each eye is composed of a cup-shaped mass (6 to 12 μ in diameter) of reddish-brown pigment spherules grasping the base of a spherical or ovoid lens.

The otocysts¹ are remarkable for their large size. They are oval sacs whose three internal diameters are 0.37, 0.36, and 0.25 mm. respectively. Their size may be better appreciated after comparison with that of the otocysts of other species (see figs. 13, 16). The largest otocysts seen while examining four large specimens of *A. marina* for this purpose were found in one about 180 mm. long, where they measure 0.07, 0.16, and 0.17 mm. along each of their three internal diameters; while in a specimen 250 mm. long the corresponding measurements are 0.13, 0.12, and 0.15 mm. (fig. 16). The nearly spherical otocysts of full-grown specimens of *A. cristata* (300 mm. long), *A. grubii* (180 mm.), and *A. ecaudata* (180 mm.) have a mean internal diameter of 0.17 to 0.18 mm., 0.16 to 0.17 mm., and 0.12 to 0.13 mm. respectively. From these figures it will be seen that the otocysts of *A. assimilis* are much larger than those of any other species. Each opens to the exterior by a narrow curved tube. The external opening is very minute, and at the bottom of a groove situated immediately in front and to the outer side of the lateral portion of the nuchal organ (fig. 22).

¹ Ehlers (1901, pp. 177, 178) has recently described these organs and their numerous spherical otoliths, consisting of a concentrically layered, evidently secreted, material. These vary in size from 0.003 to 0.03 mm. The small ones are compared to those of *A. clapedii* (the author really means *A. grubii*). The larger otoliths are often irregular, and in a few a central foreign body may be observed. The opening of the tube into the cyst is very small, and Ehlers thinks this is correlated with the character of the otoliths. He also states that the otocyst is apparently larger and its external opening nearer the brain than in *A. marina*.

The opening is close to the point of origin of the œsophageal connective, i.e. at the dorsal end of the metastomial groove, so that it is more dorsally situated than the corresponding opening in *A. marina*. The lumen of the tube is small, and in two of the four examined is almost obliterated along part of its length by approximation of the walls. In three of the tubes there are fine particles of foreign matter at one or more points. The otocyst and tube are lined with a cuticle about $3\ \mu$ in thickness. The epithelial wall of the otocyst is comparatively thin (30 to $40\ \mu$). The sense cells are not easily distinguishable, at first sight, from the supporting cells, but in one series of sections they may be distinguished by the presence of neuro-fibrillæ in the former. Each sense cell is seen to be traversed by a single fine fibril, which terminates immediately below the cuticle. These cells and fibrillæ are especially abundant in the wall of the otocyst near the entrance of the tube, and they are also present in the adjacent part of the tube. Below the epithelium is the nervous sheath, among the fibres of which occur scattered fusiform or stellate cells. The nerve-supply to the otocyst is derived from the œsophageal connectives (see above, p. 750). The otocyst contains the coagulated remains of the fluid with which it was filled in life. Among this coagulum are numerous minute spherical deeply staining granules, which are probably secreted by some of the cells in the wall of the otocyst (fig. 13). There are about forty or fifty otoliths in each otocyst; they are usually spherical, but a few are oval, and some are irregular, but have a rounded outline. They nearly all show concentric markings indicating the method of their formation by deposition of layer upon layer of a secretion produced by cells in the wall of the otocyst. The largest otoliths are $35\ \mu$ to $45\ \mu$ in diameter. In the centre of a few of them there is a minute refringent body, evidently of foreign origin, forming the nucleus around which the secreted matter has been deposited. Besides the contents already named, there are in the otocyst several deeply staining bodies varying in size from the minute granules

present in the coagulum to spherical, oval, or elongate masses $10\ \mu$ in diameter, which are either free or adhering to the surface of one of the otoliths. Their appearance suggests that they are composed of a substance similar to that of which the otoliths are formed, although the latter are usually much more lightly stained (fig. 13).

The nuchal organ of *A. assimilis* resembles that of *A. marina* in its main features. The epithelium of the organ is composed of exceedingly slender columnar cells; some of these—the sense cells—are $70\ \mu$ to $80\ \mu$ long, and only about $2\ \mu$ wide, and have deeply staining nuclei. The intervening supporting cells are a little stouter, and their nuclei stain less deeply. Many of these cells are ciliated, and some are glandular. Beneath the epithelium there is a layer of nerve elements in connection with the posterior brain-lobes. From this layer neuro-fibrillæ may be traced into and through the entire length of many of the sensory cells.

Similar fibrils may be seen in some of the epidermal cells of the general body surface, and of the papillæ of the proboscis.

III. Specimens of *Arenicoia* from New Zealand.

Three of these were collected in Otago Harbour, and are respectively 136, 126, and 90 mm. long. Another specimen from the Macquarie Islands is 217 mm. long. The Otago specimens are of a light brown colour, the two larger ones being darker in the anterior gill region, and the Macquarie specimen is dark brown throughout its length.

External Characters.—The prostomium (fig. 20), the nuchal organ, and the metastomial grooves agree in form and relations with those of *A. assimilis*. There are nineteen chaetigerous segments, of which the last thirteen usually bear gills. The first gill is thus situated on the seventh segment, as in *A. marina*, *A. clapedii*, and *A. cristata*. The gills of the two larger Otago specimens are all fully developed, but in the smallest specimen the last right gill is smaller

than any of the others, and its fellow on the left side is represented by a single filament about half a millimetre in length and bifid at the tip. In the Macquarie specimen there are only twelve pairs of gills, the first being well developed and situated on the eighth chætigerous annulus. The true first gill¹ is totally absent, a condition frequently met with in *A. marina*. The gills of the Otago specimens are of the pinnate type, and are beautifully regular in arrangement. Each consists of fourteen to sixteen main stems, usually 3.5–4 mm. long (but in several cases reaching 6 mm.), which radiate from the base of the notopodium, and are connected near their bases by a web-like membrane. Each stem bears eleven to eighteen pairs of pinnæ, which are either opposite or almost alternate in arrangement and usually divide dichotomously. These gills are remarkable for the enormous size of the afferent and efferent blood-vessels, best seen in the main stems and in the larger pinnæ. They closely resemble the gills of the Laminarian variety of *A. marina* figured by Gamble and Ashworth (1898, pl. i, fig. 2), except that the webbing at the base is more pronounced in the southern specimens. The gills of the Macquarie specimen are of a different type. They have only seven or eight main trunks 3.5–5.5 mm. long, each bearing six or eight pinnæ on each side, and these are less regularly arranged than in the Otago specimens. There is no connecting membrane at the bases of the main trunks. These gills resemble in form, but are larger than, those of the Uschuaia specimens of *A. assimilis* (see p. 740).

The annulation of the skin is exactly like that of *A. assimilis* (see p. 741).

Setæ.—The notopodial setæ (figs. 2, 2A) taper more abruptly at the tip than those of *A. assimilis*. Those of the Otago specimen are 4 mm. long. The usual pointed barbs or processes are present on one side of the shaft of the seta, while on the other is a well-marked lamina, which in most setæ is 12 μ , and in some is 15 μ broad. The

The pair of efferent vessels of this segment is also completely suppressed.

margin of the lamina is in many cases entire, but in some is very finely dentate. The setæ of the Macquarie specimen are about 5 mm. long, and the lamina is much narrower, being only about $6\ \mu$ in width.

The neuropodia are well developed; the crotchets show an interesting feature. On examining the post-rostral region (fig. 7), there are seen to be about five to seven teeth—that is, five, six, or seven teeth are in focus at the same time as the rostrum, and lie approximately in the same plane. On focusing slightly above or below this level, there comes into view a number of teeth situated on the sides of the rostrum, so that the latter projects from the centre of a series of teeth arranged around its base (fig. 8). The small subrostral process marks the position of the base of the lowest tooth of the series. Only those chætæ which have not been much worn show these lateral teeth.¹ The rostrum is slightly longer and more pointed, and the enlargement near the middle of the shaft better marked than in Ehlers' species. Many of the crotchets are strongly curved. They vary in length in the Otago specimens from 0.66 mm. to 0.8 mm., and in the Macquarie specimen they reach a length of 0.86 mm. The last-named chætæ are stouter, the rostrum more rounded at the tip, and more nearly in line with the shaft, and the teeth are more feebly marked; these characters are due to the greater age of the specimen from which the chætæ were taken.

Musculature.—The musculature is very similar to that of *A. assimilis*, except that the muscles in the region of the first diaphragm are more slender in the Otago specimens. Oblique muscles are present along the body from the first diaphragm to the end of the tail. There are no pouches on the first diaphragm.

Alimentary Canal.—The alimentary canal agrees most minutely with that of *A. assimilis*. Multiple œsophageal

¹ They are very well seen in the crotchets of post-larval stages (see fig. 10). A similar series of teeth is present around the base of the rostrum of the chætæ of other Arenicolidæ, but they are not so easily distinguished as in the specimens above described.

pouches, to the number of seven on each side, are present in the three specimens examined. The anterior pair is long and filiform or club-shaped, measuring in one case 15 mm., and in the other two specimens 24 mm. in length. The other pouches are pyriform or ovoid, and 3 to 5 mm. long.

Vascular System.—The vascular system agrees closely with that of *A. marina*,¹ and only differs from that of *A. assimilis* in the position of the first efferent branchial vessel. The first six efferent branchial vessels open into the subintestinal vessels, and the last seven into the dorsal vessel. The heart of the specimen 126 mm. long contains a moderately developed heart-body.

Nephridia.—There are six pairs of nephridia, opening, as in *A. marina* and *A. assimilis*, on the fourth to the ninth segments. In the three specimens examined the first pair is smaller than any of the others, and one of the nephridia is considerably reduced, no funnel being visible. The funnels of the first nephridia lie on the anterior face of the first diaphragm. Their dorsal lips bear about six broad, but usually undivided, ciliated processes, and their ventral lips, though small, are thrown into several of the peculiar folds or frills as described above (p. 746) in the nephrostomes of *A. assimilis*. The funnels of the other nephridia are larger; their dorsal lips bear about sixteen spatulate processes, most of which are subdivided terminally into five or six, and their ventral lips are thrown into some twenty or more folds. The vesicles of the nephridia had been recently greatly distended but are now almost empty.

Gonads.—The gonads are small and occur in the usual position. None are present on the first pair of nephridia. It is probable that the breeding season of these specimens was practically over at the time of their capture (September, 1899). It is evident that the nephridial vesicles had been recently subjected to great distension, and this was probably due to the accumulation therein of genital products. Similar

¹ Except that in the Otago specimens there is in connection with the third nephridium only one blood-vessel, viz. an afferent branch of the ventral vessel.

distension of the vesicles occurs during the breeding season in *A. marina*, *A. grubii*, and *A. ecaudata* (Gamble and Ashworth, 1898, pl. iii, fig. 15, and 1900, pl. xxvi, fig. 47). On staining and clearing the nephridia the vesicles are found to still contain either a few large ova or masses of spermatids. The ova are of the same somewhat flattened shape as those of *A. assimilis*. Their three axes measure 0.195 to 0.20 m.m., 0.16 to 0.175 mm., and 0.075 mm. respectively. (For the measurements of the ova of other species see p. 747.)

Central Nervous System.—The brain resembles that of the *Uschnuaia* specimens, except that the anterior lobes are much broader. In the specimen 126 mm. long the brain is about 0.7 mm. long, and is broadest across the anterior lobes at the point of origin of the œsophageal connectives. The breadth of the brain at this point is 1.5 mm. and its depth 0.4 mm. After the fusion of the two anterior lobes the brain rapidly narrows, so that its middle region is only about 0.7 mm. broad. The structure of the anterior lobes is exactly as described for *A. assimilis* on pp. 748, 749. Near their point of union larger ganglion-cells occur near the middle line, gradually increasing in number posteriorly and being found over the whole dorsal face of the neuropile of the mid-brain. In the posterior part of this region there are a few groups of pyriform, fusiform, or pyramidal ganglion-cells, the stout processes (usually only one to each cell) of which are united into a number of bundles. These processes pass downwards into the ventral portion of the neuropile, where they branch freely (see fig. 23). Similar ganglion-cells extend some distance into the posterior brain lobes. In other respects the brain of these specimens conforms to the description given on pp. 748, 749.

The œsophageal connectives arise, as usual, from the posterior part of the anterior cerebral lobes. They lie immediately below the epidermis of the metastomial groove, and give off numerous nerves to the skin and buccal musculature. There is a slight swelling on the connective at the origin of the nerve to the otocyst.

The situation and structure of the nerve-cord agree with the description given on p. 750. Sections taken in the mid-branchial region show one, two, or sometimes three giant-fibres. Serial sections of three segments (fig. 11) of this part show that in the first and last segments there are two giant-cells, and in the middle one only one cell. When two cells are present they lie, as in *A. grubii*, one behind the other, the anterior one being only a little distance posterior to the parapodium. The cells are laterally situated, pyriform in shape, and their single process is directed into the adjacent fibrous portion of the cord.

Sense Organs.—The nuchal organ and the reddish-brown eyes have the usual structure and position.

The otocysts are somewhat smaller and lie more laterally than in *A. assimilis*. They are almost spherical sacs (fig. 15) about 0.21 mm. in diameter, which communicate with the exterior by a tube, whose external aperture occupies a similar position to the corresponding opening in *A. marina*. It is situated near the metastomial groove, but further from the brain than in *A. assimilis*. The otoliths are of purely external origin. They consist of numerous irregular bodies (quartz-grains, fragments of spicules, etc.), without any of the chitinous covering which is usually associated with the otoliths of *A. marina*, and which forms the major portion of each otolith of *A. assimilis*. There are in each otocyst from twenty to fifty moderately large bodies, the largest being 55 μ long and 27 μ broad, and also a quantity of finer débris of similar origin and character. The lumen of the tube is slit-like, and about halfway down one of the tubes there are at two points large foreign bodies. In the structure of its wall the otocyst agrees with that of *A. assimilis* (p. 753).

IV. Systematic Position of *Arenicola assimilis* and of the Specimens from New Zealand.

Arenicola assimilis is clearly distinguished from all other species by the following characters:—(a) externally, by its twenty chaetigerous segments and the presence of the first gill on the eighth segment; and (b) internally, by the possession of six pairs of nephridia opening on the fourth to the ninth segments, by the presence of numerous œsophageal glands and of large otocysts opening to the exterior, and by the absence of the pouches on the first diaphragm.

This species obviously falls within the caudate section of the genus *Arenicola*. It has practically no points in common with *A. cristata* except those of generic value; the two species differ in every one of the characters named above. Ehlers' species has some points of resemblance to *A. claparedii*; in fact, the two most characteristic features of the latter species are found in *A. assimilis*, viz. the multiple œsophageal glands and the absence of diaphragmatic pouches. But these two species are clearly distinguished by the differences in the number of segments, the position of the first gill, the number of nephridia, and the presence in *A. assimilis* of large otocysts, such organs being absent in *A. claparedii*.

The structures hitherto believed to be diagnostic of *A. marina* are also found in Ehlers' species, viz. six pairs of nephridia opening on segments 4 to 9, and a pair of open otocysts. These two species may be easily differentiated by an inspection of the number of segments, the position of the first gill, the œsophageal glands, and the first diaphragm (to ascertain the presence or absence of pouches).

So that, although related in some degree to *A. marina* and *A. claparedii*, *A. assimilis* is quite distinct from either, and may be easily determined by reference to the six characters given above.

The determination of the systematic position of the New

Zealand specimens is a matter of considerable difficulty. In the number of chaetigerous segments and position of first gill they resemble *A. marina* and *A. claparedii*, but the prostomium is more nearly like that of the former. Internally there are four characters, two of which are in agreement with those of *A. marina* and in contrast to those of *A. claparedii*, and two are vice versa—(1) the number and position of the nephridia and the presence of open otocysts, and (2) the presence of multiple œsophageal glands and the absence of pouches on the first diaphragm. The absence of otocysts in *A. claparedii* is so remarkable and characteristic a feature that their presence in the Otago specimens, taken in conjunction with the important differences in the number of nephridia and the form of the prostomium, is sufficient to exclude the southern specimens from Levinsen's species. While their relationship with *A. marina* rests on a stronger basis, the internal differences are too important to be passed over, and one must look elsewhere for a nearer ally.

Throughout the description of the anatomy of the New Zealand specimens it is striking how frequently a perfect agreement occurs between them and *A. assimilis*. Their prostomia are practically identical, and they further agree in almost every internal character—the number and position of their nephridia, their œsophageal glands, the absence of pouches on the first diaphragm, the form and structure of the brain, the large size of their open otocysts and of their ova. The only differences are externally in the number of segments and the position of the first gill, and internally in the vessels of the seventh segment and in the nature of the otoliths. It is a question whether these differences are of sufficient importance to justify the separation of the New Zealand specimens as a distinct species.

The form of the otoliths is certainly very different. In *A. assimilis* they are rounded, and consist almost entirely of a substance secreted by the cells in the wall of the otocyst, while in the New Zealand specimens they are irregular foreign bodies (figs. 749, 751). Ehlers has re-

marked (1901, p. 178) on the small size of the opening from the tube into the otocyst in *A. assimilis*, and considered that this was connected with the form of the otoliths. I had previously arrived at the conclusion that their shape was due to the closure of the lumen of the tube, and had examined a number of otocysts of *A. marina* to obtain further evidence on this question. The anterior ends of nine specimens of the latter species have been sectioned, and show considerable differences in the character of their otoliths. Six of the specimens are comparatively young (from about 17 to 65 mm. in length), and their otoliths are irregular foreign bodies such as quartz-grains, portions of spicules, frustules of diatoms, etc., which are almost naked, i. e. they have either no secreted covering, or else it is a mere film, the presence of which is indicated by its staining with hæmatoxylin. Of the remaining three older specimens, one, which is about 170 mm. long, has irregular otoliths like those described above, but in the other two, which are about 130 and 250 mm. long respectively, the otoliths have quite a different appearance. They were at first irregular, but the original particles have been covered by layer upon layer of secreted substance, and the resultant otoliths have rounded outlines (see fig. 16). The tubes of these two pairs of otocysts are found to be practically closed along almost their whole length, either by apposition of the walls or by the blocking of the lumen by a granular substance secreted by the gland-cells in the wall of the tube. In each case the walls of the tube are so closely apposed that the lumen along the greater part of its length is reduced to a slit not more than 3 or 4 μ across, and even this space is occupied by a thin band of the secreted substance mentioned above, thus effectually closing the passage. The variation in the nature of the otoliths is probably dependent on the condition of the tube. At any rate, it is interesting to note that in the large specimen (170 mm. long) with irregular otoliths mentioned above, the lumen of the tube, as seen in section, is a fair-sized slit, and is not encroached upon to any extent by secretion

such as blocks the tubes in the specimens with rounded otoliths. It is also worthy of note that in the other species of *Arenicola* (*cristata*, *grubii*, *ecaudata*) which have rounded or spherical otoliths, formed largely of secreted matter, the otocyst is a closed vesicle. It seems probable, therefore, that the presence or absence of an open passage connecting the otocyst to the exterior has considerable influence upon the character of the otoliths, which varies even in different specimens of the same species. The fact that the otoliths of *A. assimilis* are rounded, while those of the New Zealand specimens are irregular, is not of fundamental importance; it probably indicates that in the former the tube leading from the otocyst to the exterior very soon became blocked, and the otoliths are therefore largely composed of material deposited around the small particles which had gained access to the otocyst before the closure of its tube. The otocysts of *A. assimilis* and of the New Zealand specimens agree in the most important character, namely, that each possesses a tube leading to the exterior; and the modification which takes place in the former, causing a difference in the nature and shape of the otoliths, may be regarded as of secondary importance, since a similar, though not so marked a difference, may be observed within the limits of a single species (*A. marina*). For further remarks on this subject see p. 771.

While the number of chaetigerous segments in the ecaudate *Arenicolidae* varies greatly (from about twenty-four to forty in *A. grubii*, and thirty-five to fifty-six in *A. ecaudata*), it is peculiarly constant in three of the caudate species, there being invariably nineteen in *A. marina* and *A. clapedii*, and seventeen in *A. cristata*. In most American specimens of the last-named species there is, however, an extension into the tail of structures which are usually associated only with parapodia. Small gills and cirriform processes occur upon the first two or three tail segments of one specimen examined (Gamble and Ashworth, 1900, p. 442, figs. 31, 32), and similar processes are commonly present on American specimens, but

have never been recorded in any Neapolitan specimen of this species.

With this example in mind it is not difficult to believe that, in a species probably widely ranging over the enormous coast-line of the South Atlantic and Pacific Oceans, some specimens may have become modified in the direction above indicated, so that finally a condition was reached in which some members of the species possess nineteen and others twenty segments. If we suppose that an additional parapodium and gill have been produced, the only alteration necessary to bring such a form into line with *A. assimilis* would be the loss of a gill at the anterior end of the series. The reduction and absence of the first gill are so frequently observed in *A. marina* (and to a less extent in almost all other species) that the reduction and eventual loss of the first gill of the hypothetical form are quite conceivable.

In my opinion the possession of an extra chætigerous segment, though striking, is scarcely a sufficiently important character to form by itself a test of specific value, and to be used as the sole means of distinguishing two otherwise identical forms. It seems preferable to regard the New Zealand specimens as forming a variety of the species *A. assimilis*, to which the name *affinis* may be given indicating its close connection with and resemblance to the type.

V. Post-larval Stages of *Arenicola* from the Falkland Islands.

After finding multiple œsophageal pouches in adult specimens of *Arenicola assimilis*, it occurred to me that I might be in error in the determination of the species of certain post-larval *Arenicolidae* from the Falkland Islands, and a re-examination of them became necessary. The specimens were preserved in, and handed to me in, formalin, and I examined them in that fluid two years ago. On finding multiple œsophageal glands I had little hesitation in referring them to the species *A. claparedii*, because at that

time the occurrence of several pairs of œsophageal cæca was known only in this species, and, indeed, was considered to be one of its most characteristic features. At the same time I looked for the otocysts, but did not succeed in finding them.¹ They would have been moderately easy to see in post-larval stages of *A. marina* of the same size, and finding no similar structures in my post-larvæ I therefore concluded (wrongly, as it now appears) that otocysts were absent. With these two features in mind, but relying especially on the very obvious presence of several œsophageal pouches, I identified the specimens as post-larval stages of *A. claparedii*, and as such they were recorded by Miss Pratt (1901, p. 12). The fact that these specimens had been obtained in the region in which *A. assimilis* is found had not escaped my notice, but as Ehlers' account (1897, pp. 103, 104) stated that his species closely resembled *A. marina*, it was naturally concluded that the presence of multiple œsophageal glands might still be regarded as a diagnostic character of *A. claparedii*.

These post-larvæ have now been carefully re-examined, both entire and in sections, with the result that my previous determination is found to be wrong; they are the young stages of *A. assimilis*, var. *affinis*.

The three specimens were found on the surface of the sea near the Falkland Islands, by Mr. R. Vallentin, of New Quay, and were handed over to me by Miss Pratt, of Owens College, Manchester, who was working over Mr. Vallentin's collection of Polychætes.

The specimens are 7·6, 8·7, and 11·1 mm. long respectively. The largest specimen is provided with a transparent gelatinous envelope about 1 mm. in diameter, which covers the animal, except for a distance of a little over a millimetre at each end. In general aspect these post-larvæ resemble those of *A. marina*.

¹ The nephridiopores were also examined, but on account of their minute size it was impossible to make certain of their presence or absence on the critical segment (the fourth), and therefore their number could not be definitely ascertained.

There are sparsely scattered greenish-brown pigment cells in the epidermis.

The conical prostomium bears from two to four small brownish-red eyes on each side (fig. 18). It is followed by a region divided into two by a faint groove (figs. 18, 19). The anterior portion of this region is the true peristomial segment, and in the largest specimen is itself encircled by a groove, which subdivides it into two annuli. The posterior part of the region above named corresponds to the segment bearing the minute vestigial seta in the post-larvæ of *A. marina* (Benham, 1893, p. 49). There is no trace of setæ in this segment in the post-larvæ now under consideration. In adults (see fig. 20) the region between the prostomium and first chætigerous segment is divided into four rings (see p. 741), in the third of which the œsophageal connectives unite. By comparison with these post-larvæ, it is seen that the first two rings of the adult belong to the peristomium and the other two to the first true body-somite, which, in *Arenicola*, has lost its setæ and has become fused with the peristomium.

There are nineteen chætigerous segments, in each of which crotchets and setæ may be clearly distinguished. There are two kinds of setæ present in the notopodia. The more numerous and longer ones are very similar to those of the adult (fig. 3). They are about 0.3 mm. long, and bear a lamina along about half their length. The shorter setæ, about 0.25 mm. long, are obviously laminate for a short distance on both sides (figs. 4, 5). They are almost lanceolate in shape, and drawn out into long, slender tips. Only one of these setæ is usually present in each notopodium, in which there are two to four setæ altogether. There is a tendency, more marked in the lanceolate setæ, for the lamina to break up, from the edge inwards, into fine, pointed teeth (figs. 4, 6).

The crotchets are 0.07 to 0.08 mm. long, and are distinguished by the presence of a thickening, forming an encircling ridge upon the shaft of the chæta (fig. 10). This ridge lies just below the level of the epidermis. As described on p. 756,

the teeth are not confined to the region immediately behind the rostrum, as on careful focussing they may be found also on the sides of the rostrum. There is really, therefore, a circular series of teeth from the centre of which the rostrum projects, and the subrostral process is the lowest of this series. Fig. 10 A shows the appearance of the crotchet when the rostrum is in sharp focus; in fig. 10 B the other teeth seen on focussing slightly upwards are added.

Each of the posterior tail segments is divided by slight constrictions so as to present a tri-annulate appearance. The anal segment is somewhat swollen, and the lips of the anus are crenate.

There are no gills present in any of the specimens. The blood is light red in colour (in formalin).

After staining and clearing the specimens the alimentary canal could be well seen (fig. 18). The muscular pharynx leads into the thick-walled œsophagus, which bears on the dorsal surface of its posterior portion the œsophageal glands, of which there are from six to eight visible on each side; the anterior one is the largest. Just behind this point the œsophagus is slightly constricted, and the two hearts lie on its lateral walls. The stomach is a wider tube, and upon its walls may be clearly seen the vessels of the gastric plexus bounding the chlorogogenous areas. The intestine, like that of the adult, is thrown into concertina-like folds.

Sections show that the anterior part of the œsophagus is ciliated, and that the stomach is lined by columnar cells, many of which contain a vacuole near the end which adjoins the digestive cavity.

In sections of the anterior ends of the two smaller specimens the otocysts are not easily found; they are much less clearly differentiated at this stage than those of corresponding post-larval stages of *A. marina* and *A. ecaudata*. They are seen to be two small pits in the epidermis, the lips of which are approximated so as to form a very short tube (fig. 21). Each otocyst is somewhat triangular in section; its apex is directed laterally and leads to the external open-

ing. There are in each otocyst from four to six foreign bodies (otoliths), all of which are apparently quartz-grains except two; these are obviously fragments of spicules. The otocysts of the specimen 11.1 mm. long are faintly visible in a stained preparation of the whole animal cleared in thick cedar-wood oil. They are about 40μ by 25μ in internal diameter (fig. 18).

The nuchal organ is easily recognisable; its cells are richly ciliated (figs. 18, 21).

Neither giant-cells nor giant-fibres can be identified in the nerve-cord at this stage.

Six pairs of nephridia may be traced in sections. The first nephridium is small, and its anterior end runs forward and pierces the third diaphragm. On the sixth nephridium the gonidial vessel has a covering of cells which have large spherical nuclei. This is the gonad, and it may also be distinguished, though not so clearly, on the fourth and fifth nephridia.

The above-described post-larval stages are evidently not young specimens of *A. claparedii*, as is shown by the presence of otocysts and six pairs of nephridia. They are the young stages of the variety of *A. assimilis*.

Ehlers (1897, p. 104) has recorded from *Uschuaia* a similar gill-less specimen about 6.5 mm. long, which bears nineteen chaetigerous segments. This post-larval stage was found among the "roots" of seaweeds (*Tangwurzeln*), and had probably recently settled down to its littoral habitat.

VI. Adult Specimens of *Arenicola* from the Falkland Islands.

When the foregoing account was ready for press I received, through the kindness of Mr. R. Vallentin, of New Quay, Cornwall, five adult specimens of *Arenicola* from the Falkland Islands, and have thus been able to confirm some of the observations described in the former part of this paper.

The specimens were dug from the sand in Whale Sound, Stanley Harbour, during the early months of this year (1902). They are respectively 187, 185, 135, 128, and 121 mm. long. Each has nineteen chaetigerous segments, the seventh of which bears the first and invariably small pair of gills.¹ The other external characters, e. g. the prostomium, annulation, etc., agree exactly with those of the Otago specimens, while internally the agreement is scarcely less perfect. In the two specimens dissected there are six pairs of nephridia opening on the fourth to the ninth segments. The first nephridium is small, and its nephrostome is on the anterior face of the third diaphragm. The edge of the ventral lip of the larger nephrostomes is thrown into numerous folds or frills, as figured (see fig. 17). The vascular system agrees exactly with that of the Otago specimens.

There are multiple oesophageal glands to the number of twelve or thirteen on each side, the anterior ones digitiform or club-shaped, the others pyriform or ovoid. There are no pouches on the first diaphragm.

The only feature of an unusual character in the body of the animal is the presence of a partial septum one segment behind the third diaphragm. This structure is homologous to the septa met with in the posterior branchial region of this and other Arenicolidae. It is a membrane supporting both the afferent and efferent vessels to the second pair of nephridia, and is nearly 3 mm. across in its widest part. It is not so extensive as the third diaphragm (which in the same specimen is over 6 mm. across), as it does not reach either the dorsal or the ventral body-wall. It may be regarded as merely an exaggeration of the supporting strands which are usually present in other species alongside either one or both of the vessels to the nephridia (see, for example, *A. grubii*, Gamble and Ashworth, 1900, pl. xxvi, figs. 53, 54).

The two specimens examined are females which have probably spawned, as only a very few ova are present in the

¹ In one specimen the true first gill is absent on the right side.

body-cavity. These are large, and measure across their flat faces 0.2×0.17 mm. (see pp. 747 and 758).

The anterior end of one of the specimens was cut into sections. A pair of large otocysts is present (fig. 14). They are much larger than those of the Otago specimens and a little larger than those of the worms from Uschuaia (cf. figs. 13, 14, 15). Their three diameters are respectively about 0.36, 0.38, and 0.28 mm. (compare the measurements on pp. 752 and 759).

The otoliths are all spherical or nearly so, and are composed of a yellowish or brownish secreted substance. There are in each otocyst two otoliths (fig. 14) considerably larger than the rest. They are about 0.055 mm. in diameter, and in the centre of each is a small irregular foreign body, probably a quartz-grain. The smaller otoliths are usually from 0.02 to 0.03 mm. in diameter, and only rarely is a foreign particle visible in them, though doubtless each has a very minute central nucleus of this description. The two large otoliths described above are probably the first otoliths of the post-larval stage, which always remain distinguished by their greater size from those which are formed later. A similar condition exists in *A. ecaudata*, in the post-larval stage of which there is for some time only one otolith, which always remains conspicuous, owing to its larger size (Gamble and Ashworth, 1900, p. 504 and fig. 64).

The otocyst opens to the exterior by a tube, the external opening of which corresponds in position to that of the Otago specimens and of *A. marina*. The lumen of the tube is of moderate size along the greater part of its length, but is reduced near its entrance to the otocyst in one case to a very narrow passage, and in the other is practically obliterated. The wall of the tube is remarkable for the presence of large gland-cells, which are practically confined to the dorsal wall. They are almost ovoid in shape, and their cell-contents are in the form of a reticulum. In the ventral wall of the tube there are numerous elongate fusiform sense cells.

The remaining structures shown in sections of the anterior

end are so exactly similar to those of the Otago specimens that no further description of them is necessary.

The specimens above described are interesting from their bearing on the discussion regarding the taxonomic value of the shape of otoliths (see p. 761). The only difference between the Falklands specimen and those from New Zealand is that in the former the otoliths are spherical and composed almost entirely of a secreted substance, while in the latter they consist of irregular foreign bodies, such as sand-grains and fragments of spicules. There can be no doubt that the two sets of specimens belong to the same species, or rather to the same variety, so that (as was also proved for *A. marina*, see p. 762) the shape of the otoliths varies in different specimens of the same species or variety. The closure of the tube of the otocyst along part of its length and the presence of the numerous large gland-cells in its wall are probably the principal factors in determining the shape and nature of the otoliths of the Falklands specimens. Having proved the presence of spherical otoliths in some examples of *A. assimilis*, var. *affinis*, it will be noticed that one of the differences (discussed on pp. 761—763) between this new variety and the type of the species disappears; so that now the only features by which they may be distinguished are (1) the presence of twenty chætigerous segments in the type of the species, whereas the new variety possesses only nineteen, and (2) the slightly different position of the external opening of the otocyst. As the latter is too fine a character for ready application in systematic work, it may be said that the determination rests upon the number of chætigerous segments.

Another striking feature about the otocysts is the great difference in their size in specimens of the variety from the two localities. Whereas in the Otago specimens their average diameter is 0.21 mm. (in a specimen 136 mm. long), in one (128 mm. long) from the Falklands their average diameter is 0.34 mm. (cf. figs. 14, 15), so that the internal volume of the latter is about four times that of the former.

Adult specimens of the new variety are now recorded from

the same locality as the post-larval stages described on pp. 764—768. There can be no doubt that the latter are stages in development of the former. Judging from Ehlers' record (1897, p. 104) of the capture near Uschuaia of a gill-less specimen 6.5 mm. long with nineteen chaetigerous segments, it seems probable that the variety occurs at this place along with typical specimens of the species.

VII. Distribution of *Arenicola assimilis*.

Ehlers (1901, p. 178) records the occurrence of *A. assimilis* in collections from the Straits of Magellan (Punta Arenas and Susanna Cove), the Beagle Channel (Uschuaia and Lapataia Nueva), South Georgia, Chile (Schmarda), Kerguelen (Grube), and California.

Schmarda's (1861, pp. 51, 52) *A. piscatorum* from Chile and Grube's (1878, pp. 511, 554) *A. piscatorum*, Cuv., var., from Kerguelen, are both included by Ehlers under the species *A. assimilis*. Although Schmarda gives a brief description of some points in the anatomy of his specimens he unfortunately does not mention any characters which enable their identity to be definitely settled. With respect to Grube's specimen from Kerguelen the only information given is that most of the branchiferous segments are divided into only four annuli, and owing to this feature Grube distinguished his specimens as a variety of *A. piscatorum*. There is no evidence to show that any of these specimens belong to the species *A. assimilis*.

Ehlers (1897, p. 104) states that in the Göttingen collection there is a species of *Arenicola*¹ from California in which there are twelve pairs of gills which agree in position with those of *A. assimilis*, and these specimens are dis-

¹ I thank Professor Ehlers for sending to me by letter the further information that this is a duplicate from Professor Agassiz's collection, which was sent to Göttingen to be worked over. The rest of the specimens were returned to Professor Agassiz, and are doubtless those referred to on the next page.

tinguishable from *A. marina* only by this character. No mention is made of other features which would have been much more valuable as diagnostic characters, but the difference in the number of gills is accepted as a sufficient ground for separating the specimens from *A. marina*, notwithstanding the well-known liability to reduction (from thirteen to twelve pairs) in the number of branchiæ in this species. As will be seen from the discussion below, it is very probable that Ehlers' specimen does not belong to either of these species, and that this is an example of the confusion due to placing an implicit reliance on the value of external features in discriminating species of *Arenicola*. On such a variable and insufficient character as the number of gills Ehlers bases his diagnosis of the Californian specimen, and refers it to the species *A. assimilis*. This is the only evidence in support of his record of this species from California.

I have recently re-examined specimens of *Arenicola* from a collection made by Professor Agassiz, near Crescent City, California, sent to Dr. Gamble and myself from the Harvard Museum, and identified by us (1900, p. 423) as *A. claparedii*. These specimens are the more interesting because they are accompanied by a label¹ indicating that they have passed through the hands of Professor Ehlers, and that he considered them to belong to a new species nearly related to *A. marina* (= *piscatorum*). It is almost certain that these are the same specimens which Ehlers has recorded as *A. assimilis*. There are five specimens, in three of which there are twelve pairs of gills, the first situated on the eighth chætigerous segment. In each of the other two specimens there are twelve gills on the left side (the first being on the eighth segment), accompanied in one case by thirteen gills on the right, the first being very small and borne on the seventh segment, while on the right side of the other

¹ The writing upon the label, which is now faint, is as follows:—
“*Arenicola*, n. sp. nahe *piscator*. 7 vor Segm. 12(13) Kiementrag.
Californien (E. Ehlers).”

specimen there are only eleven gills, the first of which is on the ninth segment. It may therefore be said that it is usual to find the first gill in these specimens on the eighth segment as in *A. assimilis*. Dissections of two of the specimens show that there are five pairs of nephridia, multiple œso-ageal glands, and no pouches on the first diaphragm; and sections of the anterior end prove conclusively that there are no otocysts. All these points are so characteristic of *A. claparedii* that there can be no doubt that the specimens belong to this species.

I am indebted to Dr. H. P. Johnson for two specimens of *Arenicola* from Puget Sound, Washington. In one of these¹ there are thirteen pairs of gills, but in the other the seventh segment bears a gill only on the right side, the first left gill being on the following segment. Dissections of the specimens and sections of the anterior end of one of them fully confirm the determination of their species made by Dr. Johnson (1901, p. 421); they are undoubted *A. claparedii*.

It is therefore highly probable that Ehlers is in error in recording *A. assimilis* from California. In the first place, his determination of the species of the Californian specimens rests solely upon a character which is very variable and almost useless for distinguishing species; secondly, a re-examination of what are probably the very same specimens proves them to be *A. claparedii*, and this species has been recorded from another point on the west coast of the United States.

A revision of Ehlers' record of the distribution of *A. assimilis* therefore becomes necessary, and may be given as follows:—Adult typical specimens of *A. assimilis* have been recorded from several places in the extreme south of the

¹ It is remarkable that of the seven specimens examined from the west coast of the United States this is the only one which possesses the full number of gills. On the contrary, it is unusual to find any departure from the normal number in Neapolitan specimens of *A. claparedii*; out of thirty-nine examined only two show a reduction in the number of gills; in each case there are thirteen on the left side, but only twelve on the right.

American continent and from South Georgia. Others forming a new variety but agreeing with the type, except in the number of chætigerous segments, are now recorded from Otago Harbour, the Macquarie Islands, and the Falkland Islands. Post-larval stages of the variety have been obtained off Stanley Harbour (East Falkland) and near Uschuaia.

VIII. Specific Characters of the Caudate Arenicolidæ.

Appended is a revised summary of the characters of the caudate Arenicolidæ, which clearly shows by what features, both external and internal, *A. assimilis* may be readily recognised and distinguished from other species with which it is liable to be confused. It cannot be too strongly urged that attention should be directed by systematists chiefly to internal characters in the discrimination of the species of *Arenicola*. No determination of *A. marina*, *A. claparedii*, or *A. assimilis* can be considered complete or entirely trustworthy which relies solely on external characters. It is impossible to distinguish these three species with certainty unless reference be made to the nephridia, œsophageal glands, and otocysts, the two former being of especial use in this connection.

The characters¹ of the caudate Arenicolidæ may be briefly stated thus:

A distinct tail present; the parapodia and gills do not extend to the posterior end of the animal. The body is often swollen anteriorly. Gills, pinnate or derivable from the pinnate type, eleven to thirteen pairs, the first (which may be small or even absent) on the seventh or eighth chætigerous segment. Prostomium consisting of a median and two lateral lobes. Nephrostomes with dorsal lip well provided with flattened, spatulate, ciliated, vascular processes;

¹ The following is a revision of a part of the summary published by Dr. Gamble and myself (1900, p. 540), to which reference may be made for the characters of the genus and of the ecaudate species.

ventral lip ciliated, entire (i. e. not deeply notched as in the ecaudate Arenicolidae). Gonads small, ova discoidal.

(a) *A. marina*, Linn.—Nineteen chaetigerous segments. Thirteen pairs of gills; the first, which is on the seventh segment, may be reduced (or suppressed). Otocysts opening to the exterior. Otoliths, numerous foreign bodies (quartz-grains, etc.), which may, however, be covered with a layer of secreted chitinous substance, giving them a rounded outline. Six pairs of nephridia opening on segments 4 to 9. One pair of œsophageal pouches, cylindrical, club-shaped, or conical. Diaphragmatic pouches (on the first diaphragm) small, globular, or flask-shaped.

Found on both sides of the North Atlantic.

(b) *A. assimilis*, Ehlers.—Twenty chaetigerous segments. Thirteen pairs of gills, the first of which is situated on the eighth segment (the first gill is liable to be reduced or suppressed). Otocysts large, opening to the exterior. Otoliths numerous; spherical or rounded chitinous bodies. Six pairs of nephridia opening on segments 4 to 9. Several pairs of œsophageal pouches; the anterior pair long, club-shaped, or filiform; the others much smaller and pear-shaped. No pouches on the first diaphragm.

Recorded from the extreme south of the American continent.

(c) *A. assimilis*, var. *affinis*, Ashworth.—Nineteen chaetigerous segments. Thirteen pairs of gills, the first (liable to reduction or suppression) on the seventh segment. Otocysts large, opening to the exterior. Otoliths numerous, and composed either of foreign bodies (quartz-grains, etc.) or of spherical chitinous bodies. Other characters as in the type of the species (see above).

Recorded from Otago Harbour, New Zealand, the Macquarie Islands, the Falkland Islands.

(d) *A. claparedii*, Levinsen.—Nineteen chaetigerous segments. Thirteen pairs of gills, the first on the seventh segment (this pair of gills is liable to reduction or suppression, especially in specimens from the west coast of North

America). Lateral lobes of prostomium well developed. No otocysts. Five pairs of nephridia opening on segments 5 to 9. Two or more pairs of œsophageal pouches, the anterior pair long and slender or club-shaped, the others shorter, usually pyriform. No pouches on the first diaphragm.

Recorded from the Mediterranean and from the west coast of the United States.

(e) *A. cristata*, Stimpson.—Seventeen chætigerous segments. Eleven pairs of gills, the first on the seventh segment. Otocysts, closed spherical sacs each containing a single large, spherical, chitinoid otolith. Six pairs of nephridia opening on segments 5 to 10. One pair of œsophageal pouches cylindrical or club-shaped. Diaphragmatic pouches (on the first diaphragm) large and finger-shaped.

Found in the Mediterranean, in the West Indies, and on the eastern shores of North America south of latitude 40° N.

IX. Summary of Results.

1. The anatomy of *Arenicola assimilis* is fully described for the first time. Although Ehlers states that the species differs from *A. marina* only in the number of chætigerous segments (nineteen in the latter, twenty in the former), in the position of the first gill and in the relative size of the middle lobe of the prostomium, further examination shows that there are other important points of difference, e. g. *A. assimilis* possesses multiple œsophageal glands and has no pouches on the first diaphragm.

2. Specimens of *Arenicola* are described from Otago Harbour (New Zealand) and from the Macquarie Islands which differ from the type in the number of chætigerous segments (nineteen) and situation of the first gill. There is also a difference in the shape of the otoliths; in the type they are spherical or rounded, while in the New Zealand specimens they are irregular. These specimens belong to a new variety (var. *affinis*) of the species.

3. In the discussion of the systematic position of the Otago specimens it is concluded that the form of the otoliths is not sufficiently reliable to form a character by which species may be discriminated. In *A. marina* the otoliths are usually irregular, but two out of nine specimens examined possess rounded otoliths. In these cases the otoliths were at first irregular foreign bodies, but they have been covered with layer upon layer of secreted substance, and now have a rounded outline. In each of these two cases the tube which placed the otocyst in communication with the exterior has become either wholly or partially blocked, either by apposition of its walls or by the secretion into the lumen of a glandular substance which forms an effectual plug. In the seven specimens of *A. marina* with irregular otoliths the tubes are not closed in this way. It is concluded that the presence of spherical or rounded otoliths is associated with the closure of the tube, and it is pointed out in support of this conclusion that the other species (*cristata*, *grubii*, and *ecaudata*) in which spherical otoliths are found have closed otocysts (pp. 761—763).

4. The brain is well developed. The ganglion-cells of its middle region are large (especially in the Otago specimens) and send processes into the neuropile, where they branch freely.

5. Giant-fibres and segmentally arranged giant-cells are present in the nerve-cord. They have the same structure as in *A. grubii*.

6. The otocysts of *A. assimilis* are distinguished by their size. They are considerably larger than those of any other species. Neuro-fibrillæ may be traced from the nervous sheath of the otocyst into and along the whole length of the sense cells of the otocystic epithelium. These cells and fibrils are especially abundant near the point of entrance of the tube to the otocyst.

7. There is a large nerve-supply to the skin and proboscis. Neuro-fibrillæ may be seen in some of the cells of the general body-surface and of the papillæ of the proboscis.

8. Post-larval specimens of *A. assimilis*, var. *affinis*, are described from the Falkland Islands. They possess an achæitous segment between the peristomium and first chætigerous segment (as in similar stages of *A. marina* and *A. ecaudata*). By comparison with the adult the limits in the latter of the peristomium and achæitous body-segment may be determined (figs. 19, 20).

9. Adult specimens of *A. assimilis*, var. *affinis* are also described from Stanley Harbour, East Falkland. They are remarkable for the large size of their otocysts, the internal volume of which is about four times that of the otocysts of the Otago specimens (figs. 14, 15). It is evident that a considerable variation in the size of these organs may occur in specimens of the same species or variety from different localities. The otoliths, several of which contain an irregular foreign body, are spherical, and in one specimen two of them are much larger than any of the others. They are the first two otoliths of the post-larval stage which have continually received fresh depositions of secreted substance, and always remain distinguished from those formed later by their larger size. These specimens from the Falklands differ from the Otago specimens in the nature of their otoliths. Here is additional evidence that the character of the otoliths contained in otocysts provided with a tube leading to the exterior is not a feature upon which much value should be placed in systematic work. Blocking of the tube (as occurs in the Falklands specimen) converts the otocyst into a closed sac, in which spherical otoliths are formed, while in other specimens (e. g. those from Otago Harbour) in which the tube remains open the otoliths are irregular foreign bodies, such as sand-grains, which are able to gain access to the otocyst.

10. Ehlers records *A. assimilis* from the Straits of Magellan, the Beagle Channel, South Georgia, Chile (Schmarda), Kerguelen (Grube), and California. It is shown that there is no evidence in support of the last three records. Schmarda's and Grube's specimens are insufficiently

described, and no character is mentioned by which their species may be determined. Ehlers' diagnosis of the Californian worms rests solely upon a character which is very variable and almost useless for distinguishing species. A re-examination of what are probably the same specimens proves them to be *A. claparedii*, and this species has also been recorded from another point on the west coast of the United States (pp. 772—774). Incidentally it may be mentioned that specimens of *A. claparedii* from the west coast of North America almost invariably bear only twelve gills either on one or both sides, while Neapolitan specimens have usually the full number (thirteen pairs).

A. assimilis may be regarded as the characteristically southern species of the genus. Adult typical specimens are recorded from several points in the extreme south of America and from South Georgia. A new variety (var. *affinis*), differing from the type only in the number of chætigerous segments, is now recorded from Otago Harbour (New Zealand), the Macquarie Islands, and the Falkland Islands. Post-larval stages of the variety have been taken off the Falklands and near Uschuaia, in the Beagle Channel.

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EXPLANATION OF PLATES 36 & 37,

Illustrating Dr. J. H. Ashworth's memoir on "The Anatomy of *Arenicola assimilis*, Ehlers, and of a New Variety of the Species, with some Observations on the Post-larval Stages.

LIST OF REFERENCE LETTERS.

A. B. S. Achæitous segment of body between peristomium and first chætigerous segment. *Ant. Cer. L.* Anterior lobe of brain. *Bl.* Bladder of nephridium. *B. V.* Blood-vessel. *C.* Cuticle. *Ch. Seg. 1* First chætigerous segment. *Cæl.* Cælom. *Conn. Tiss.* Connective tissue. *Epid.* Epidermis. *E. T.* Entrance to tube leading from otocyst to exterior. *Ext. Op. Ot.* External opening of otocyst. *Gang. C.* Ganglion-cell. *Gast. Lat.* Lateral gastric vessel. *Gl. C.* Gland cell. *Gon.* Gonad. *Gon. V.* Gonidial vessel. *Ht.* Heart. *Met. Gr.* Metastomial groove. *Middle Comm.* Middle commissure of brain. *M. Circ.* Circular muscles. *M. Long.* Longitudinal muscles. *Mo.* Mouth. *M. Sh. Bucc.* Muscular sheath of buccal mass. *N.* Band of nerve-fibres from prostomial epithelium to brain. *N. Aff.* Afferent vessel of nephridium. *N. Bucc.* Nerve to buccal mass and papillæ of "proboscis." *N. C.* Nerve-cord. *N. Epid.* Nerve to epidermis. *Nm. Ch.* Neuropodial chætæ. *Not. S.* Notopodial setæ. *Nphm. D.* Dorsal lip of nephrostome. *Nphm. V.* Ventral lip of nephrostome. *Npile.* Neuropile. *Nuc. Gr.* Nuchal groove. *Oc.* Eye. *Æ.* Œsophagus. *Æ. Conn.* Œsophageal connective. *Æ. Gl.* Œsophageal gland. *Ot.* Otocyst. *Oth.* Otolith. *Ot. T.* Tube of otocyst. *Pap.* Papilla of "proboscis." *Per.* Peristomium. *Ph.* Pharynx. *Post. Cer. L.* Posterior lobe of brain. *Prost.* Prostomium. *Prost. Epith.* Epithelium of prostomium. *Prost. Lat.* Lateral lobe of prostomium.

Prost. Mid. Middle lobe of prostomium. *Sp.* Fragment of spicule. *St.* Stomach. *V. V.* Ventral vessel.

PLATE 36.

FIG. 1.—Distal half of a seta from the fifth notopodium of a specimen of *Arenicola assimilis* from Uschuaia. $\times 100$.

FIG. 1 A.—A portion of the seta more highly magnified. Note the lamina on the left bearing fine teeth. $\times 750$.

FIG. 2.—Distal portion (two fifths) of a seta from the sixth notopodium of a specimen of *A. assimilis*, var. *affinis*, from Otago Harbour. $\times 100$.

FIG. 2 A.—A portion of the same seta more highly magnified. Note the broad lamina crossed by fine oblique lines. $\times 500$.

FIGS. 3, 4, 5.—Notopodial setæ from a post-larval specimen (7.6 mm. long) of *A. assimilis*, var. *affinis*. Most of the setæ are of the kind shown in Fig. 3, but in each notopodium there is one seta of the type seen in Fig. 5. Setæ of the kind shown in Fig. 4 are much less common than the preceding; only two specimens were seen in ten notopodia. The lamina is breaking up on one side near its tip into fine teeth. $\times 320$.

FIG. 6.—Tip of a seta of the same kind as shown in Fig. 5. Note the fine teeth on the margin of the lamina on one side. $\times 600$.

FIG. 7.—A crotchet from the fifteenth neuropodium of an Otago specimen (var. *affinis*). $\times 100$.

FIG. 8.—The head of a crotchet from another Otago specimen, to show the teeth situated on the sides of the rostrum. $\times 280$.

FIG. 9.—A crotchet from the fifteenth neuropodium of *A. assimilis* from Uschuaia. $\times 100$.

FIG. 10.—Two crotchets from the sixteenth neuropodium of a post-larval specimen (7.6 mm. long) of *A. assimilis*, var. *affinis*. *A* shows the appearance of the chæta when the rostrum and post-rostral teeth are in focus; in *B* the teeth on the sides of the rostrum are also shown. The subrostral process is now seen to be one of the series of teeth. The dotted line indicates the level of the epidermis. $\times 600$.

FIG. 11.—Diagram of a portion of the nerve-cord of *A. assimilis*, var. *affinis*, from Otago Harbour, to show the distribution of the giant nerve-cells. The transverse lines indicate the position of the neuropodia, the numbers of which they bear. The nerve-cord is magnified about 10 times and the cells 40 times.

FIG. 12.—Diagram of a portion of the nerve-cord of *A. assimilis* from Uschuaia, to show the distribution of the giant nerve-cells. The transverse lines indicate the position of the neuropodia. The last giant-cell shown

situated (probably) in the first tail segment. The nerve-cord is magnified about 10 times and the cells 40 times.

FIG. 13.—Shows the size of the otocyst of a specimen (120 mm. long) of *A. assimilis* from Uschuaia. The oval outline is a camera drawing of the cuticle which lines the otocyst. The tube which leads from the exterior enters the otocyst near the point marked *E. T.* The otoliths are rounded, and many of them show concentric markings, indicating their deposition in layers. In the centre of several of them small foreign bodies may be distinguished. Attached to some of the otoliths are other small rounded bodies of a similar nature, but which stain more deeply. The minute deeply staining spherules (indicated by the dots) are also composed of a similar substance. $\times 210$. (Cf. Figs. 14, 15.)

FIG. 14.—Camera drawing of the cuticle lining the otocyst of a specimen (128 mm. long) of *A. assimilis*, var. *affinis*, from the Falkland Islands. The otocyst is somewhat larger than the one shown in Fig. 13. The otoliths are nearly spherical. (They are not all present in one section; some are added from another section.) The two larger ones are probably the first otoliths of the post-larva, which are easily distinguished by their size from those which are formed later. In the centre of each of the large otoliths an irregular foreign body may be seen. $\times 210$. (Cf. Figs. 13, 15.)

FIG. 15.—Camera drawing of the cuticle lining the otocyst of a specimen (136 mm. long) of *A. assimilis*, var. *affinis*, from Otago Harbour. The otocyst is much smaller than either of the two preceding. The otoliths are irregular bodies, chiefly quartz-grains, but two small fragments of spicules (*Sp.*) are seen lying close together. $\times 210$. (Cf. Figs. 13, 14.)

FIG. 16.—Camera drawing of the cuticle lining the otocyst of *A. marina* (about 10 inches long). The tube connecting this otocyst to the exterior is almost blocked, and in consequence the otoliths, which were originally small irregular foreign particles, are now assuming a rounded outline, due to the deposition upon them of layer upon layer of secreted substance; see, for example, the otolith containing the spicule fragment (*Sp.*). Note the small size of the otocyst compared to those shown in Figs. 13, 14, and 15. $\times 210$.

FIG. 17.—Fifth nephridium of *A. assimilis* (specimen from Punta Arenas). The dorsal lip of the nephrostome (*Nphm. D.*) bears the usual ciliated vascular processes, while the edge of the ventral lip (*Nphm. V.*) is thrown into numerous folds or frills. Note the gonad, a somewhat club-shaped mass of cells around the gonidial vessel (seen by transparency through the gonad). $\times 10$.

FIG. 18.—Left aspect of the anterior portion of a post-larval specimen of *A. assimilis*, var. *affinis*, from the Falkland Islands. The total length of this specimen is 11.1 mm. Note the four cup-shaped eyes on the prostomium. Between the prostomium and the first chætigerous segment is a region

imperfectly divided into two by a groove; the anterior part—the peristomium (*Per.*)—is again partially subdivided into two annuli, in the anterior of which the otocyst (*Ol.*) may be faintly seen; the posterior part (*A. B. S.*) is the first body-segment, which, however, does not bear any traces of setæ. The succeeding segments bear notopodial and neuropodial setæ. The former usually consist of two or three capilliform bristles and one lanceolate seta (see also Figs. 3—6). The alimentary canal is seen by transparency through the body-wall. The buccal mass leads into the œsophagus, which dilates in the fourth chætigerous segment, and in the sixth bears the glands (*Æ. Gl.*), seven of which may be recognised. Immediately behind this the œsophagus is contracted, and leads into the stomach, on whose surface the almost rectangular chlorogogenous areas are already differentiated. The nephridiopores are not shown, as they are too minute to be detected with certainty. $\times 50$.

FIG. 19.—The anterior end of the same specimen. Ventral aspect. The slit-like cœlomic cavity between the two anterior brain-lobes is seen by transparency in the prostomium. The metastomial field—the area included between the œsophageal connectives—is slightly raised above the level of the general epidermis. The specimen shows the peristomium (*Per.*), the achæitous body-segment (*A. B. S.*), and the first chætigerous segment (*Ch. Seg.*). See description of previous figure. Compare these parts with those of the adult shown in Fig. 20. $\times 50$.

FIG. 20.—The anterior end of an adult specimen of *A. assimilis*, var. *affinis*, from Otago Harbour. Dorsal aspect. Note the prostomium with its V-shaped lateral lobes embracing the median one. The nuchal groove (*Nuc. Gr.*) and the origin of the metastomial groove (*Met. Gr.*) are shown. The first chætigerous segment (*Ch. Seg.*) consists of three annuli. The region between this and the prostomium is divisible into two almost equal parts, an anterior part—the peristomium (*Per.*)—in which the two annuli are not very regular, and a posterior part, consisting of two annuli, which form the achæitous body-segment (*A. B. S.*). $\times 8$. (Cf. Fig. 19.) The form of the prostomium and the annulation of the skin of type specimens of *A. assimilis* from Ushuaia are exactly the same as shown in this figure.

FIG. 21.—Transverse section of a post-larval specimen of *A. assimilis* var. *affinis*, 8.7 mm. long. The section passes somewhat obliquely through the posterior brain-lobes, the nuchal organ, and the otocyst of the right side. The two posterior cerebral lobes (*Post. Cer. L.*) are seen in the prostomium separated by a portion of the cœlomic cavity. They are closely applied to the nuchal organ, the ciliated epithelium of which is well seen on the left, but is cut obliquely on the right. The right otocyst is seen as an invagination of the epidermis, the mouth of the pit being narrowed to form the tube of the otocyst. Already a few foreign bodies (otoliths) have gained admittance. Note the large gland-cells scattered in the epithelium, the sections of the

œsophageal connectives, the pharynx, the circular and longitudinal muscles. The nuclei of the muscle-fibres have been omitted. $\times 210$.

PLATE 37.

FIG. 22.—Transverse section of the anterior end of a specimen of *A. assimilis*, from Uschuaia. The section passes through the anterior part of the brain at the point of origin of the œsophageal connectives. The two anterior cerebral lobes are seen in the prostomium in close relation to its epithelium; bands of nerve-fibres (*N.*) may be seen passing from the latter into the brain. The cells in the brain are represented by the dots shown in the figure. The spaces seen in the brain and in the subepidermal tissue are canaliform prolongations of the cœlomic cavity. On the left the section has passed somewhat obliquely through the skin, and shows the numerous branches given off from the œsophageal connective, and ending in the basal part of the epidermis. The dots in these nerves represent the nerve-cells which are present. Immediately to the left of the prostomium is the external opening of the otocyst (*Ext. Op. Ot.*), the tube being cut through along a considerable part of its length; a transverse section of the inner part of the tube is seen just ventral to this. In the lower part of the figure the buccal mass is seen cut across; the elevations of the epithelium (*Pap.*) shown are the papillæ of the “proboscis.” The nerves (*N. Bucc.*) which supply these are shown. Note the strong musculature of the buccal mass. $\times 30$.

FIG. 23.—Transverse section of the middle portion of the brain of a specimen of *A. assimilis* var. *affinis*, from Otago Harbour. Only the left half of the section is drawn; the median plane is indicated by the two vertical lines. In the upper part of the section the intimate relation of the prostomial epithelium and the brain may be observed; the ganglion-cells extend up to, and lie among the bases of, the epithelial cells. The dots in the brain represent the nuclei of small nerve-cells, which are usually arranged in groups or clusters. The large cells in the middle of the figure show the form and position of the larger ganglion-cells of the brain. Their processes extend downwards into the neuropile, where they branch. Note the numerous fibres passing from the left half of the brain across the middle line to the right, forming the middle cerebral commissure. The isolated cell on the right side is drawn from another section. $\times 210$.



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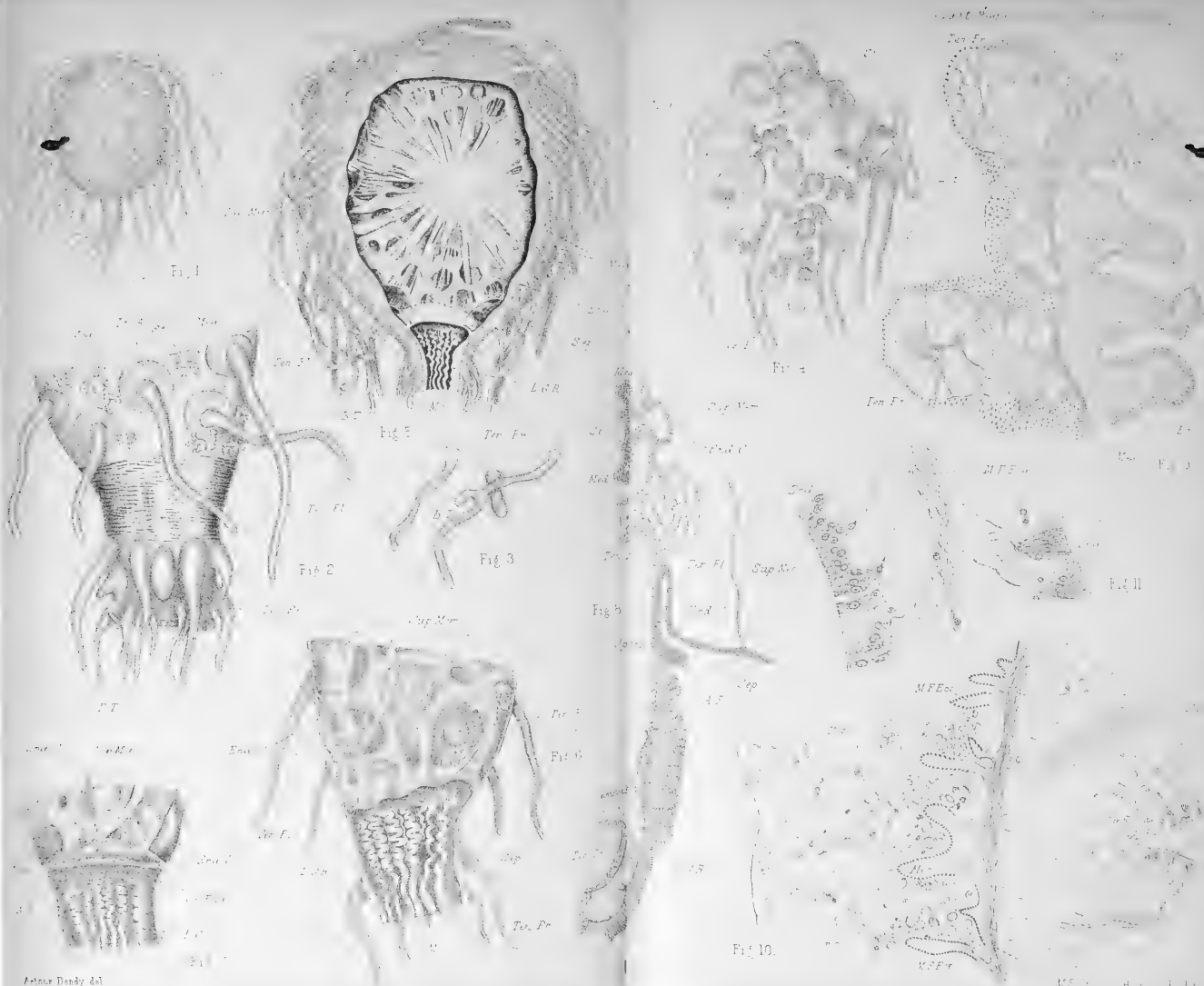
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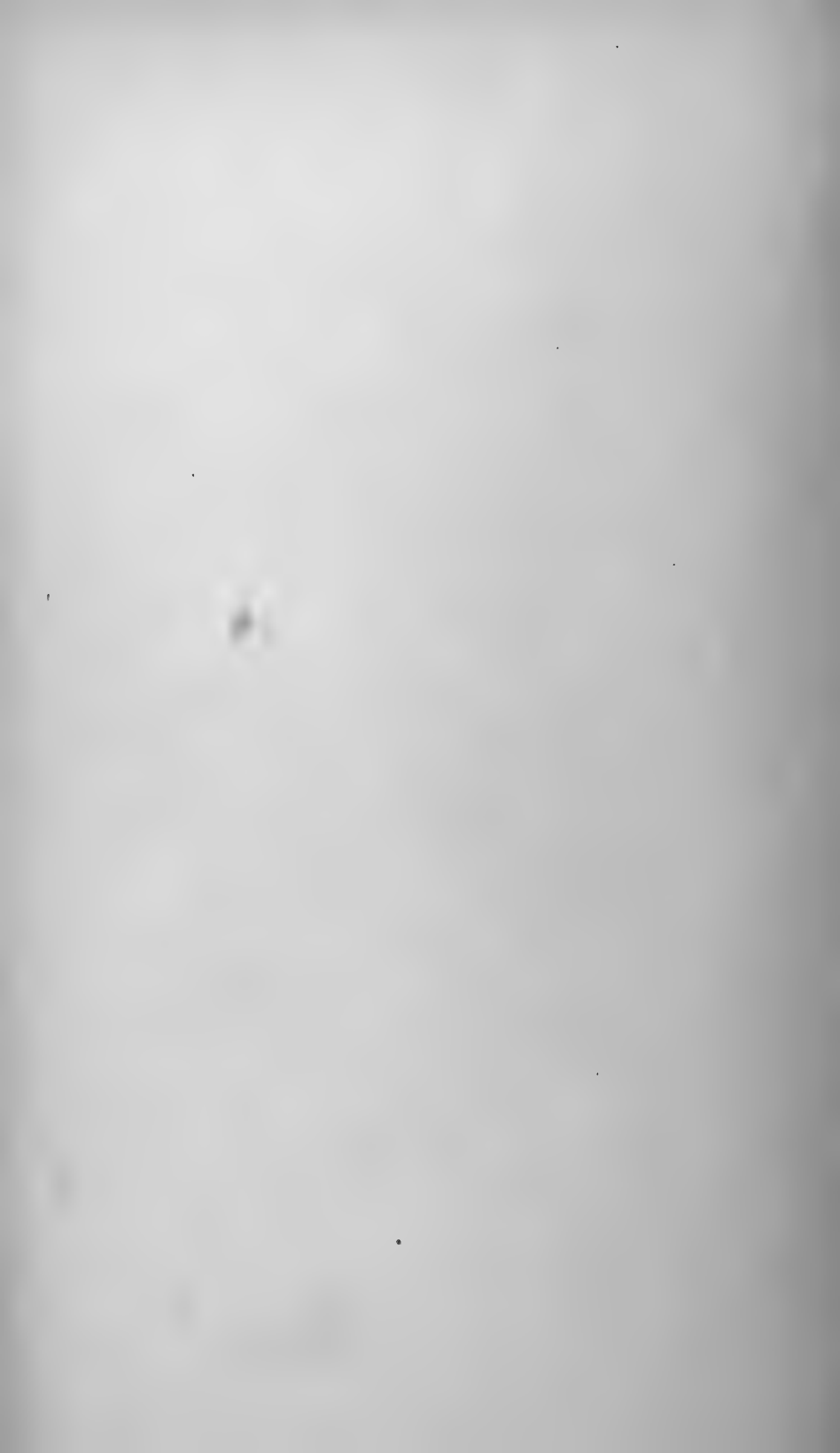
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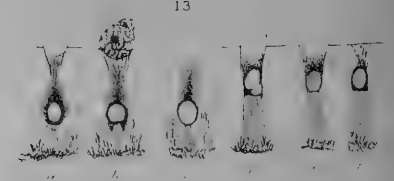
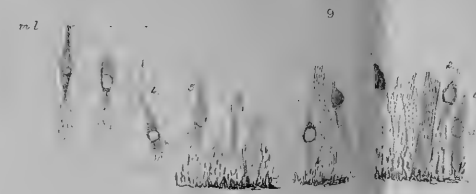
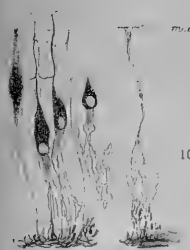
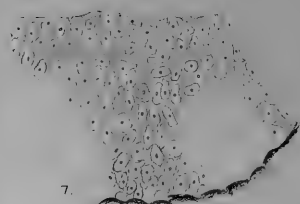
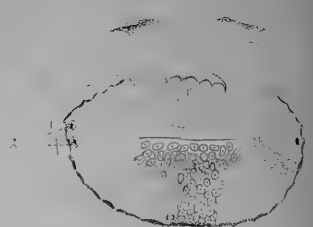
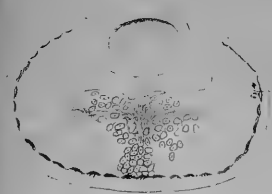
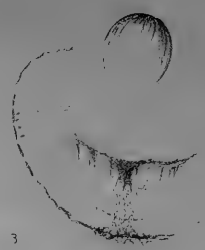
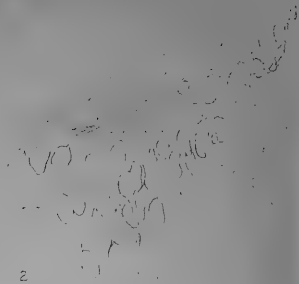
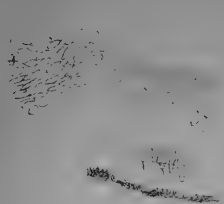
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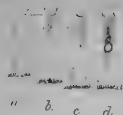












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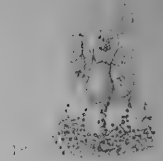
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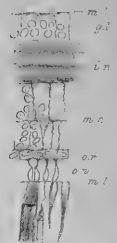
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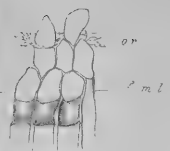
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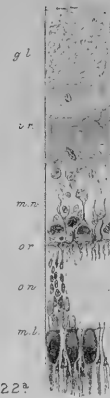
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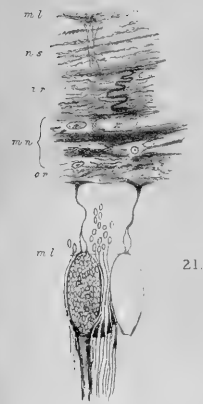
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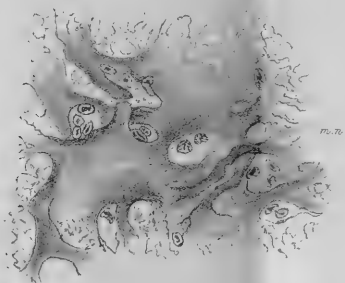
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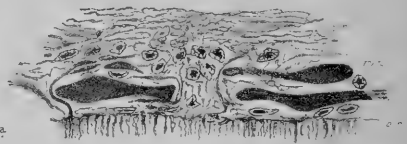
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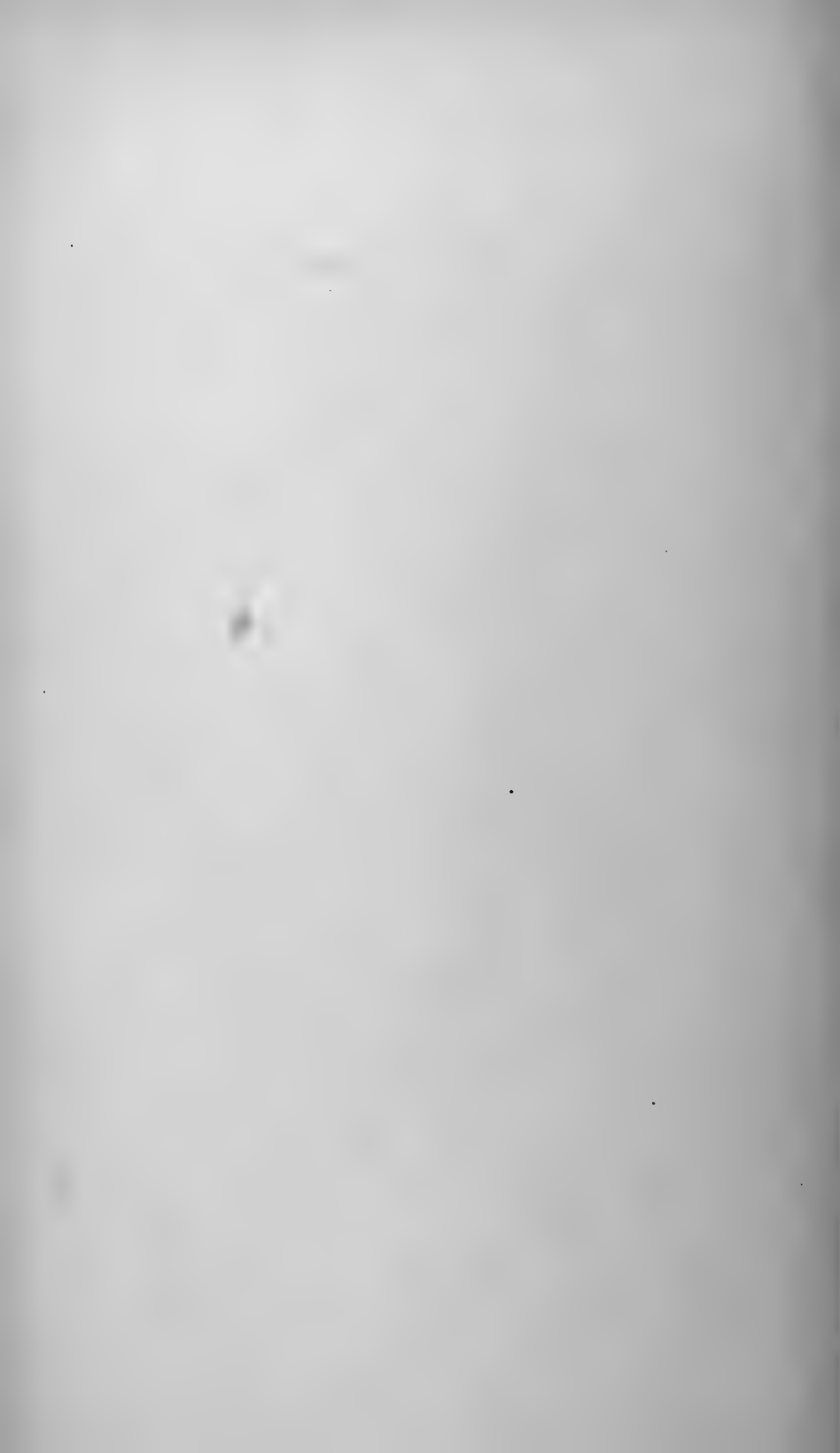
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23^a



23^b



24^a

24^b

25^a

25^b

25^c

25^d

25^e

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30

27

28^a

28^b

28^c

29^a

29^b

32^a

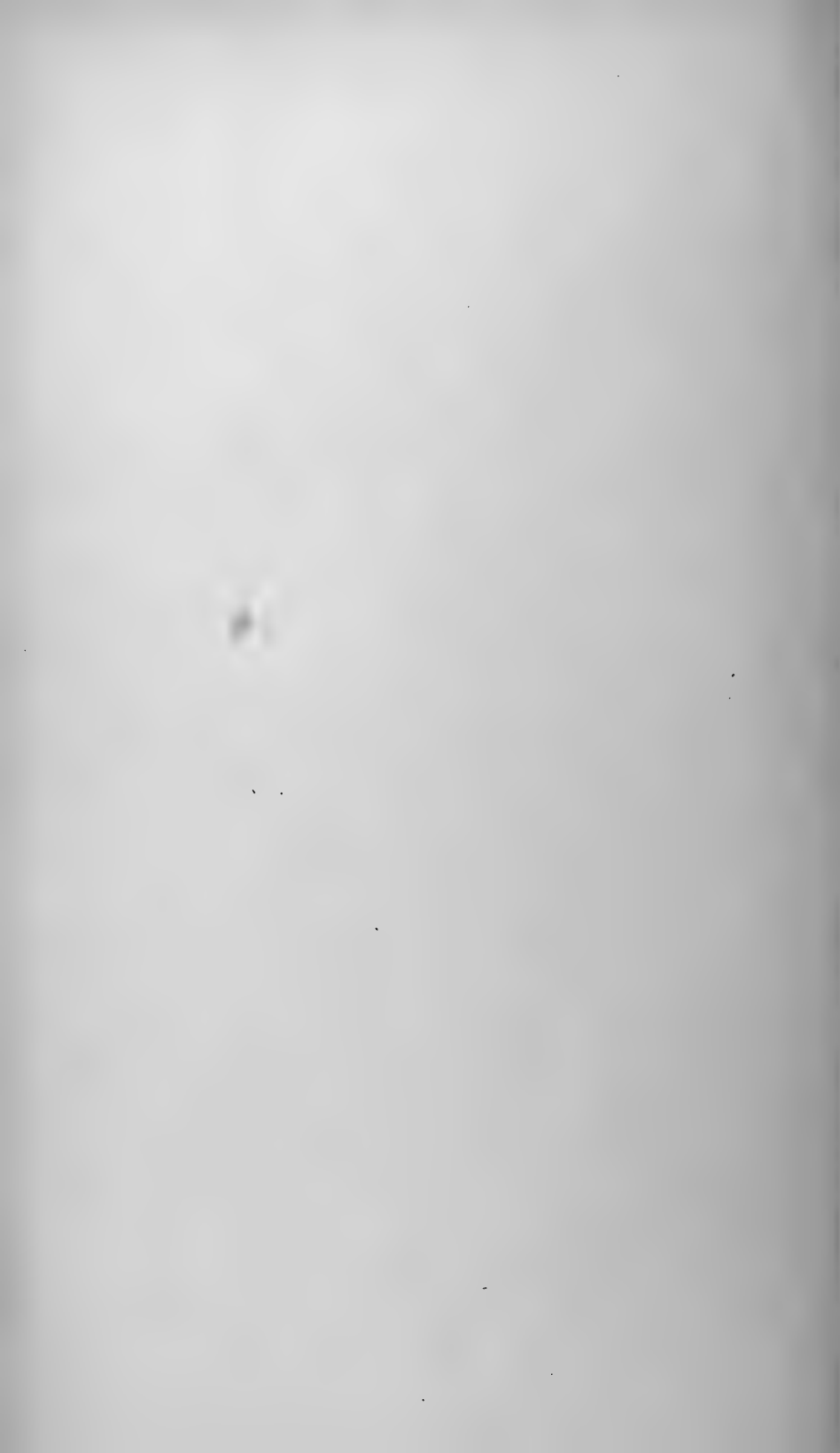
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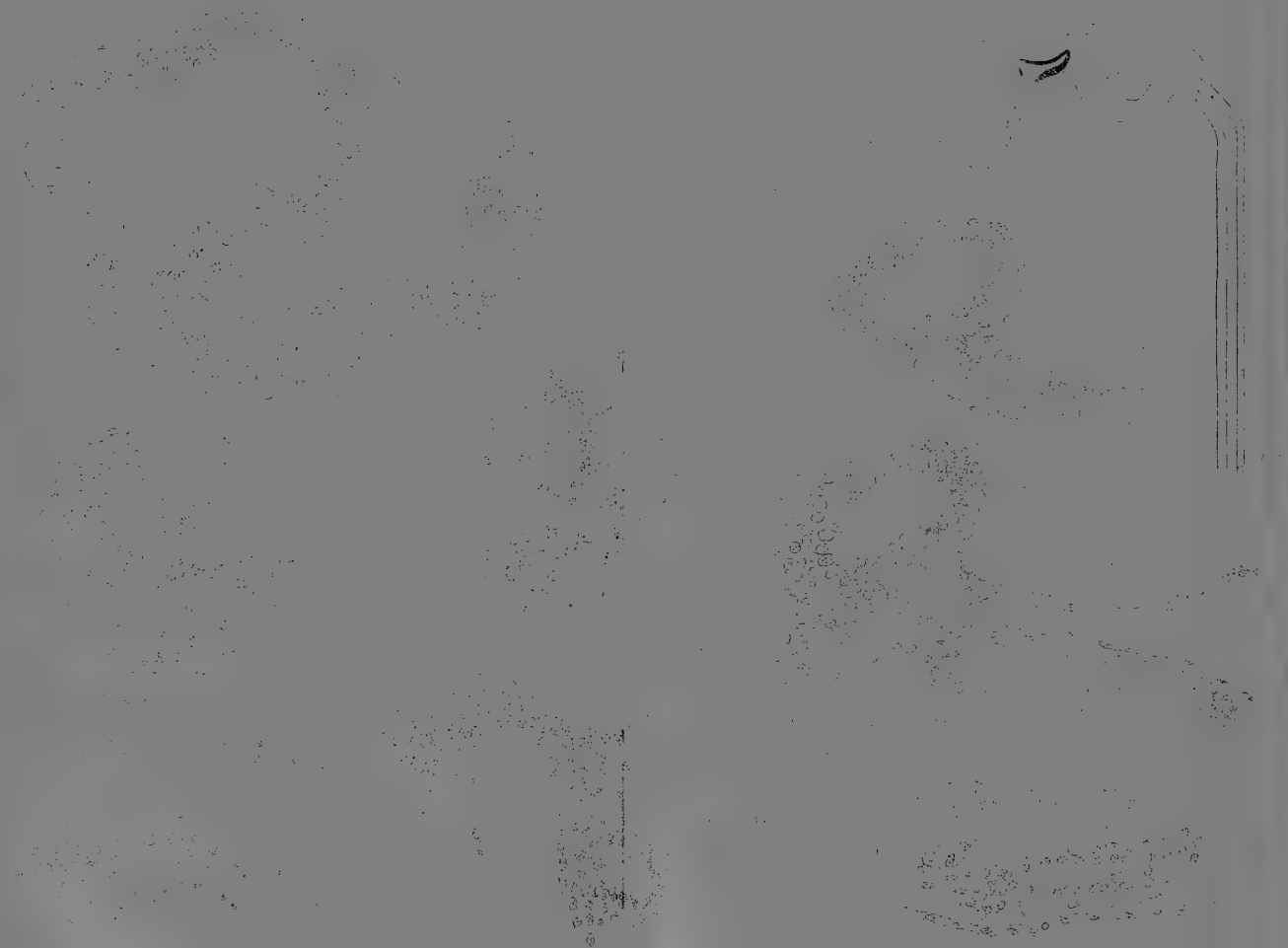
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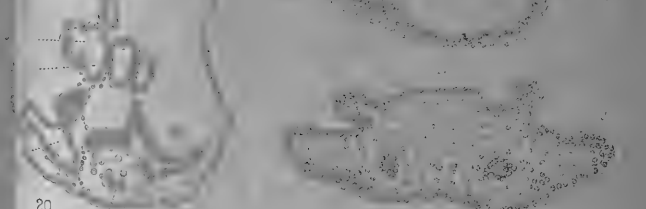
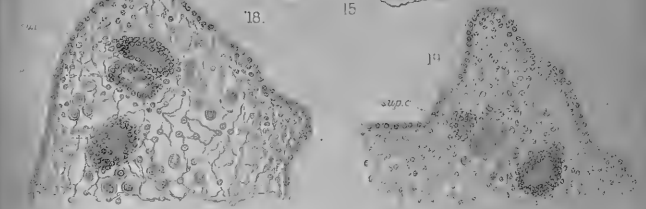












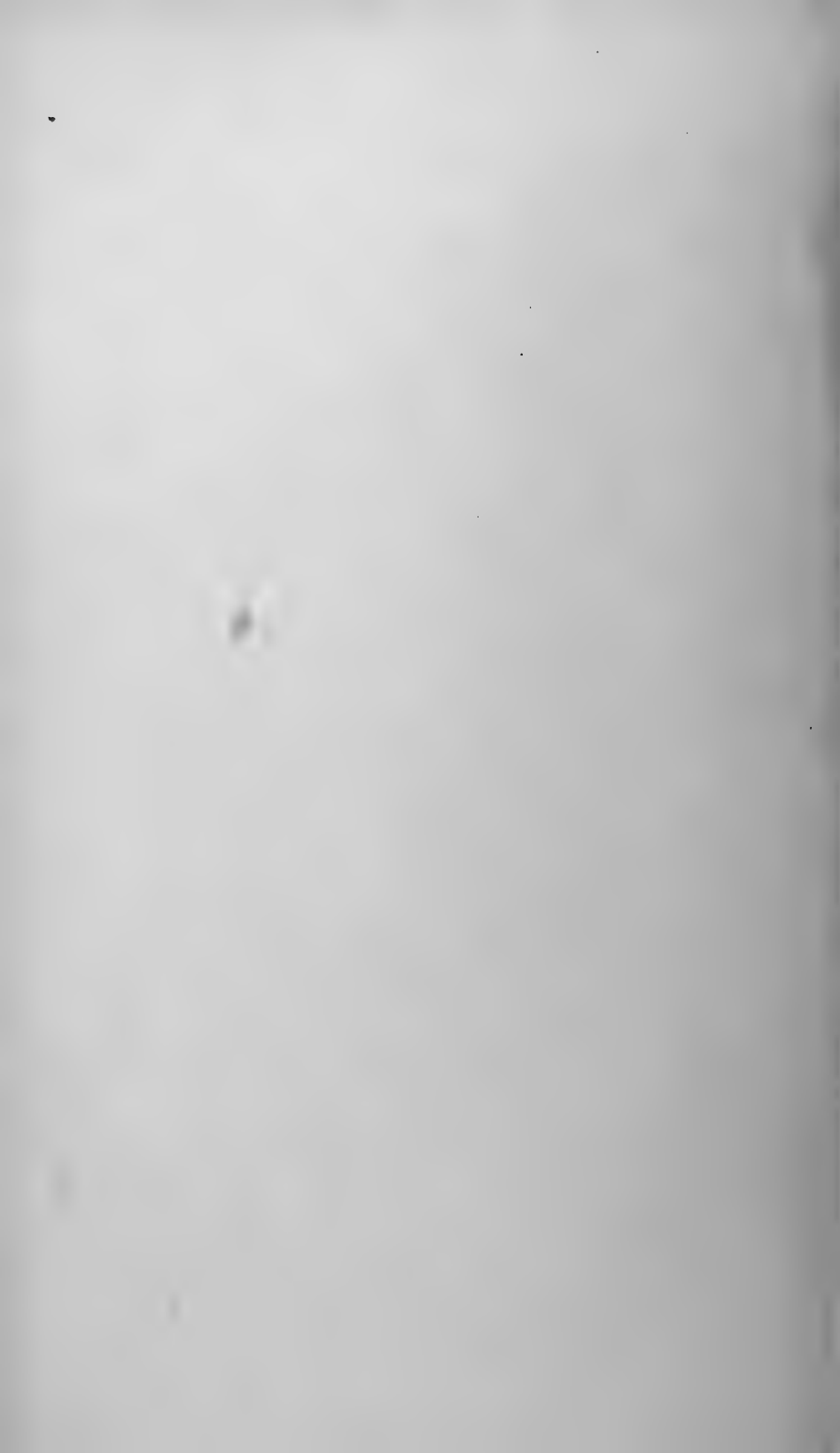






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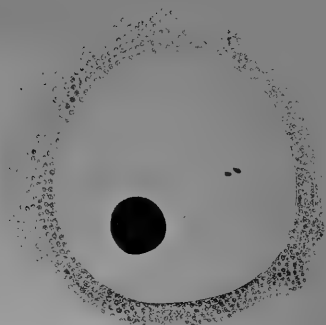


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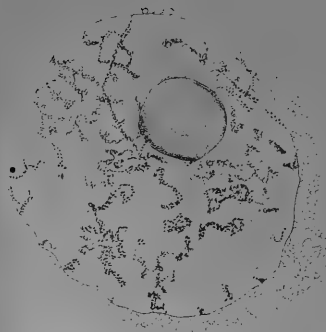


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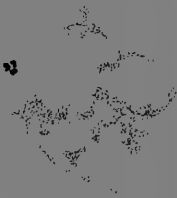


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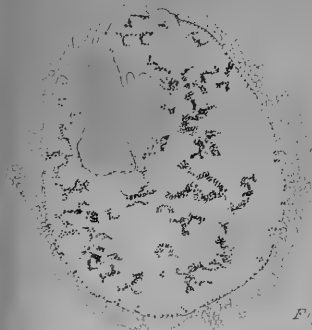


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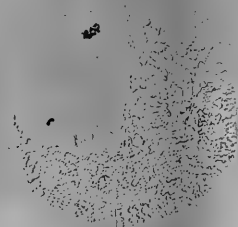


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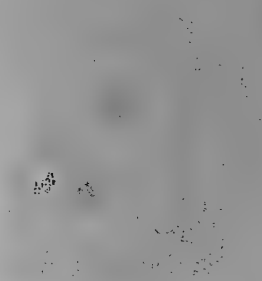


Fig. 7



Fig. 8

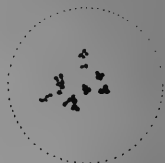


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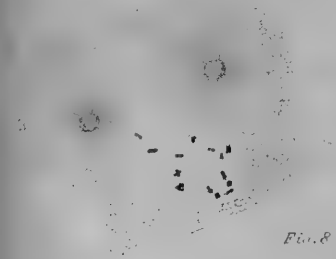


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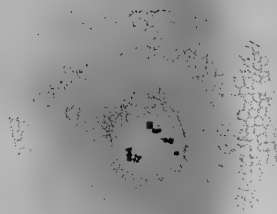


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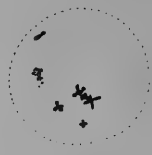


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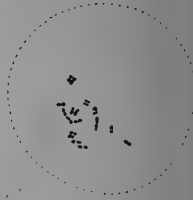


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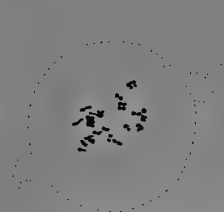
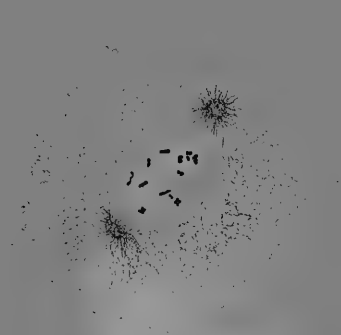


Fig. 13

Fig. 14



For 15

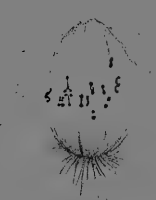


Fig 17

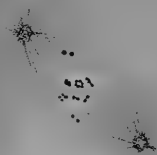
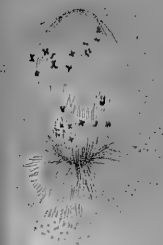


Fig. 16



Fig. 18'



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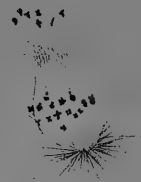


Fig. 20



Profile views.

Fig. 21

Face views

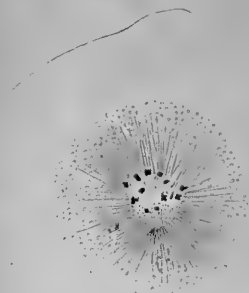


Fig. 22.



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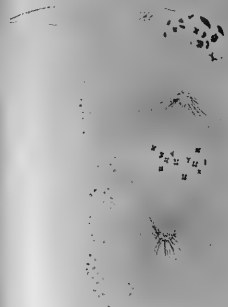


Fig. 24



Fig 25



Fig. 26

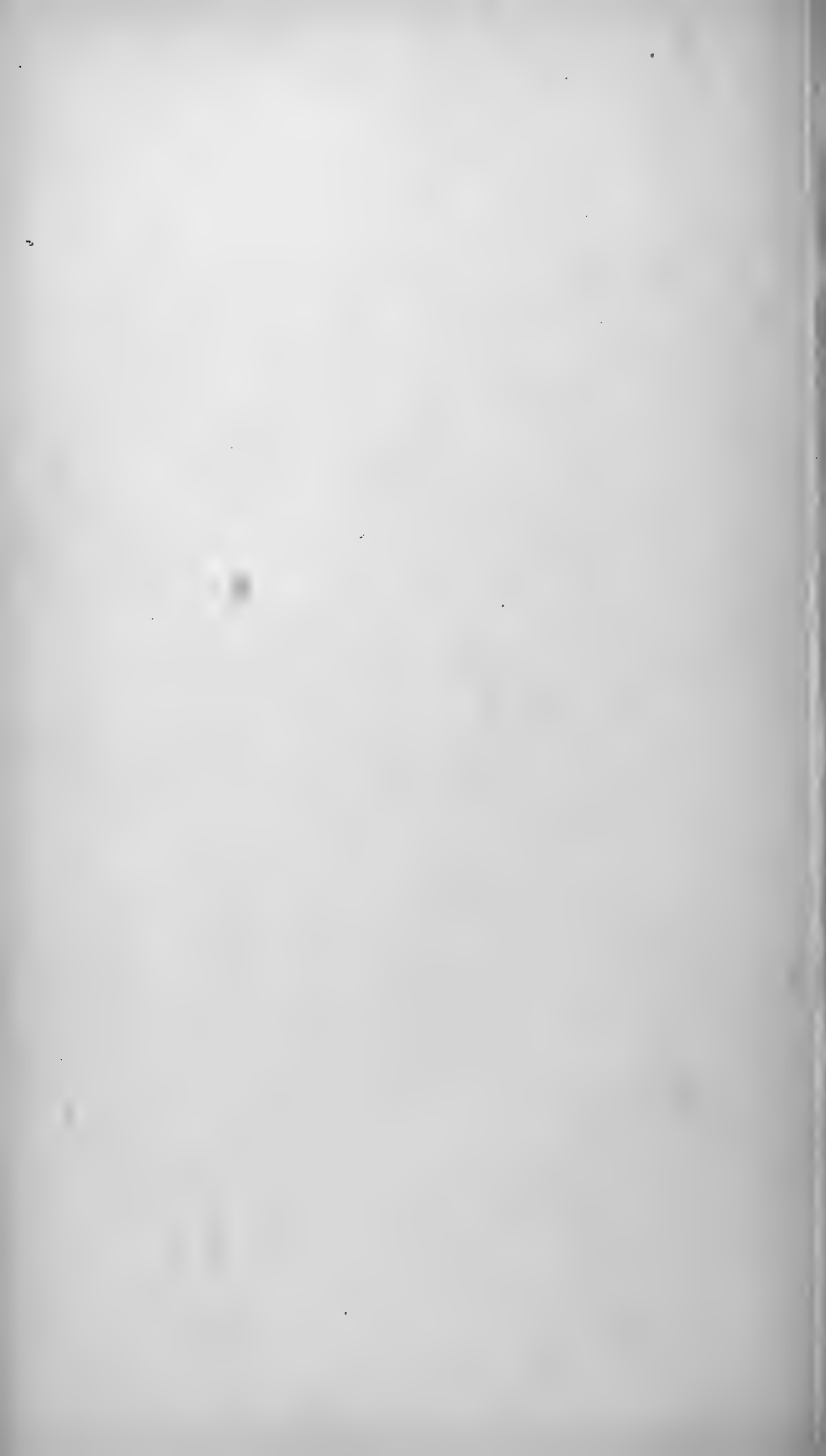




Fig. 27.



Fig. 28.



Fig. 29.

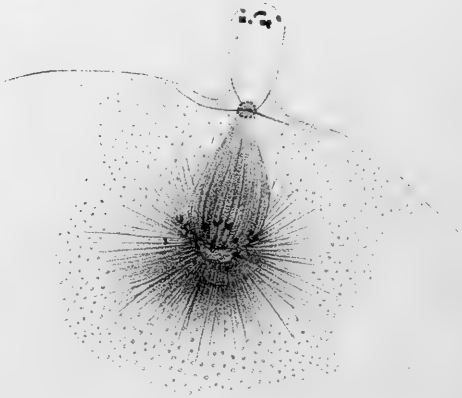


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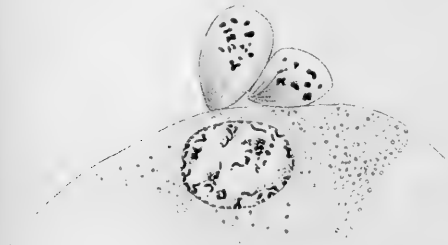


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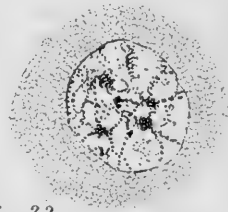


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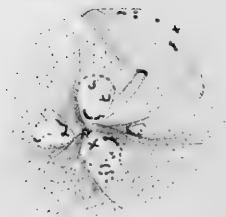


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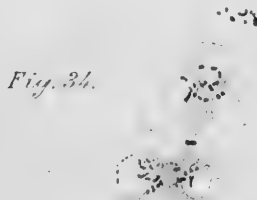
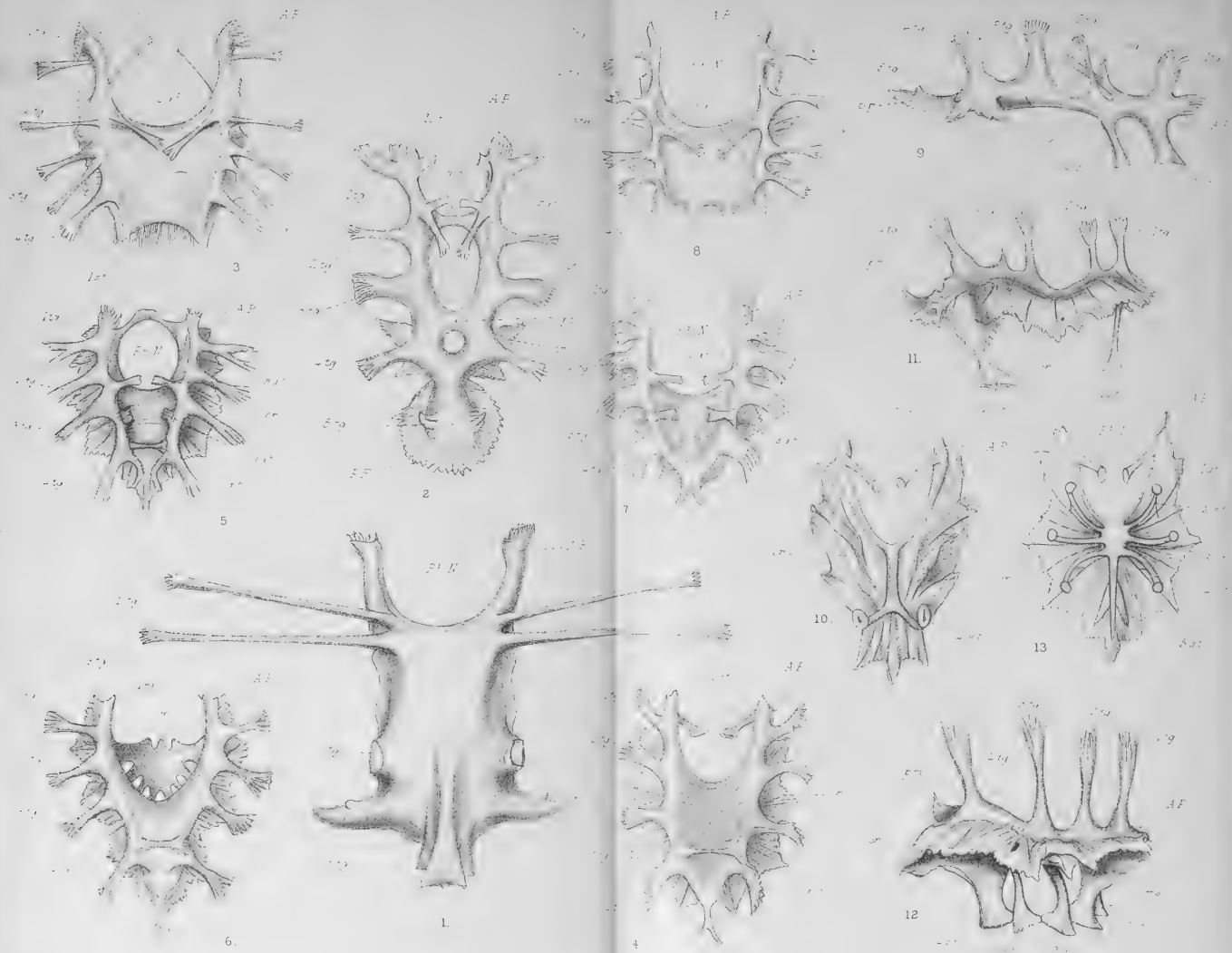
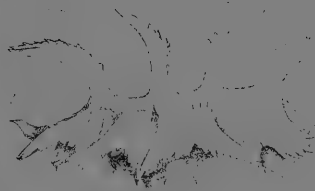


Fig. 34.









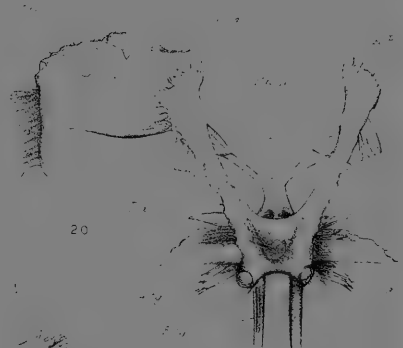
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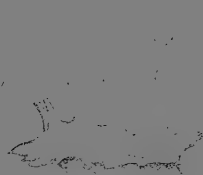
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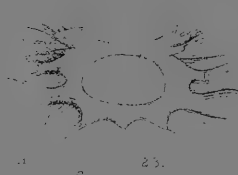
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Fig. 1

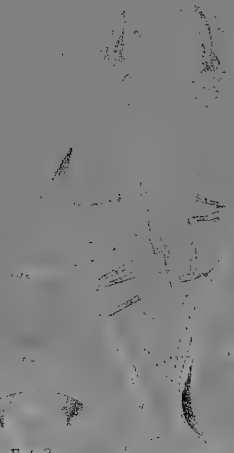


Fig. 2



Fig. 3

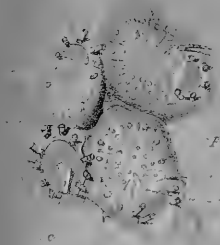


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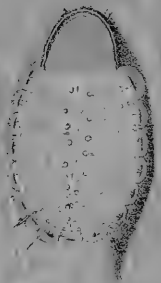


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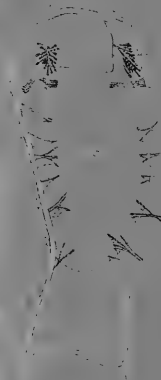


Fig. 6



Fig. 7

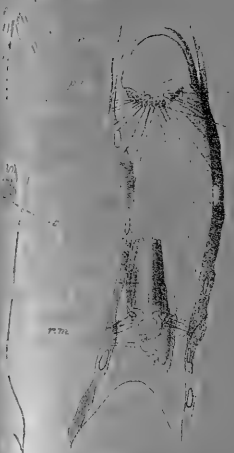


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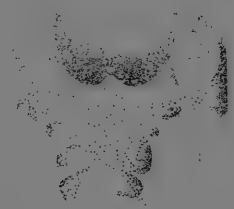


Fig. 9



Fig. 10



Fig. 11



Fig. 12



Fig. 13



Fig. 18.

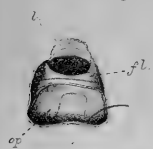


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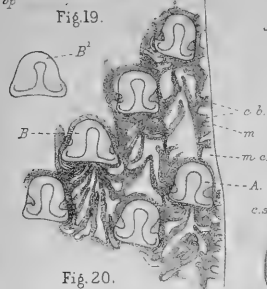


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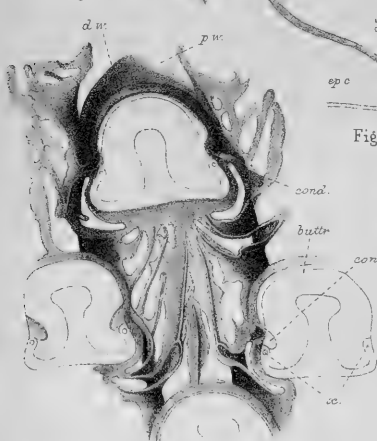


Fig. 21.

Fig. 23.



Fig. 24.



Fig. 25.

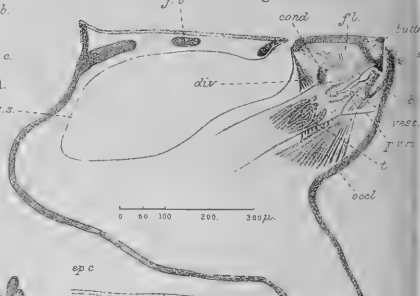


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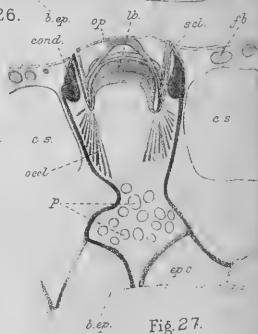


Fig. 27.

Fig. 28.



Fig. 29.



Fig. 30.



Fig. 31.

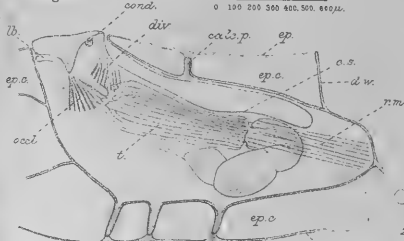


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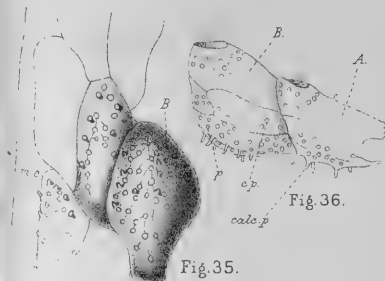


Fig. 35.

Fig. 32.

0 100 200 300 400 500 600 μm.

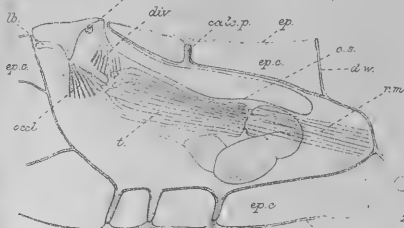


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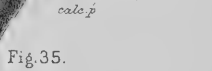


Fig. 33.

0 50 100 200 300 400 500 600 μm.



Fig. 37.



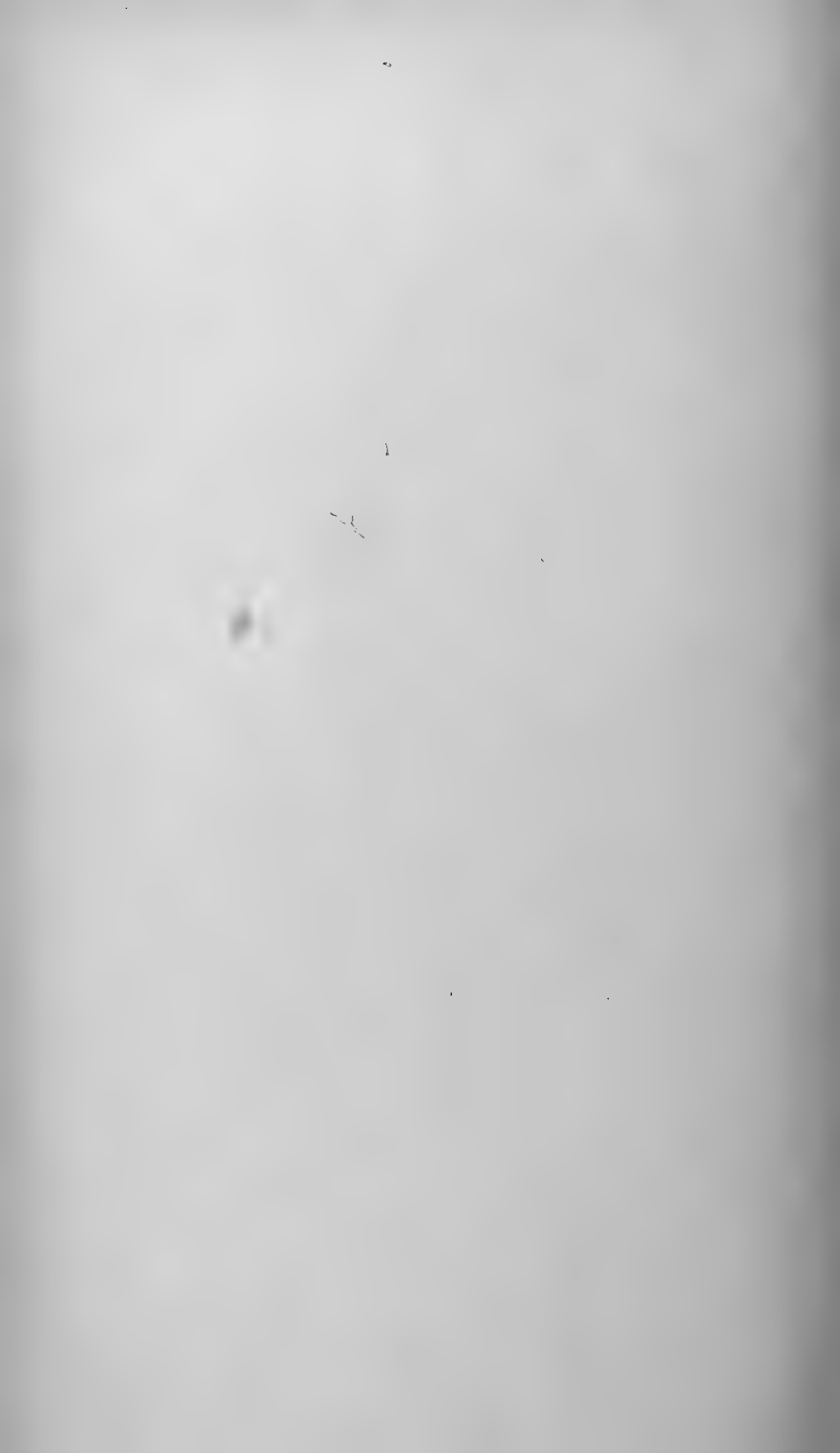


Fig 38

Fig 41

Fig 45

Fig 47

Fig 49

Fig 40.

Fig 42

Fig 46.

Fig 48.

S.E. Harner del.

Fig 39.

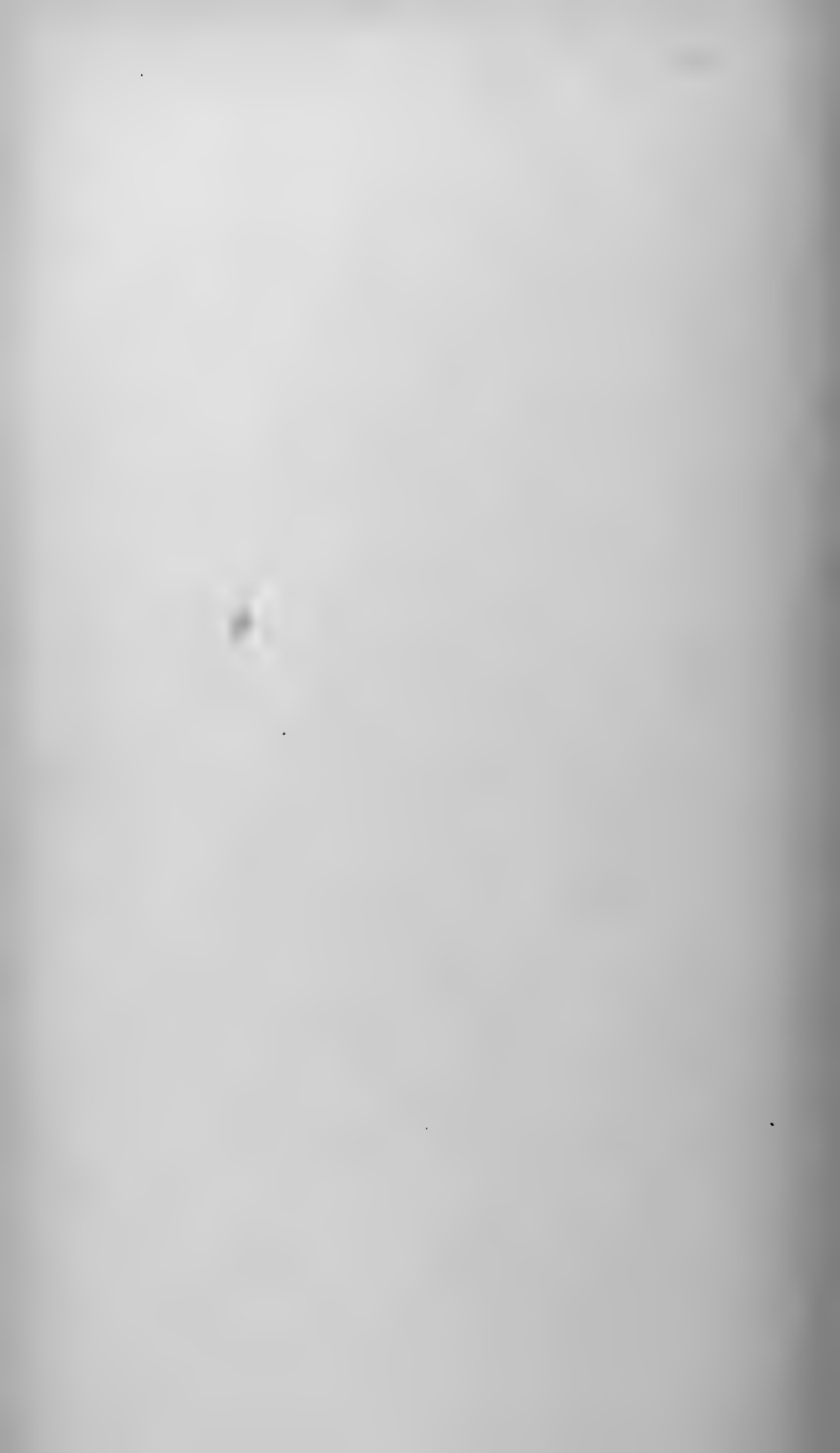
Fig 43.

Fig 44.

Fig 50.

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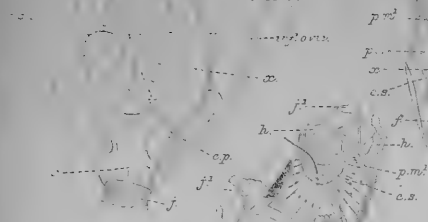


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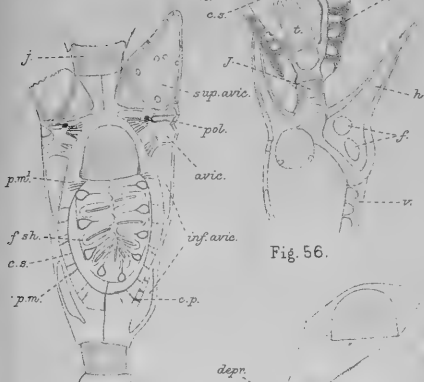


Fig. 56.



Fig. 55.



Fig. 57.

S.F. Warner del.



Fig. 58.



Fig. 59.



Fig. 60.

0 50 100 200 300 400 μ

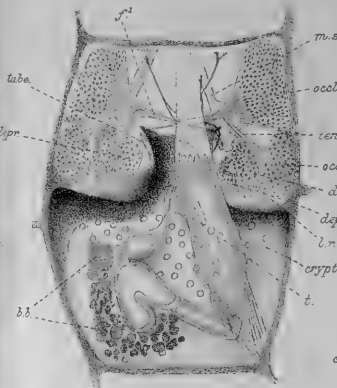


Fig. 61.



Fig. 62.

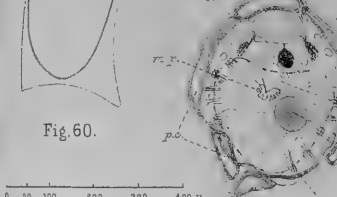


Fig. 63.

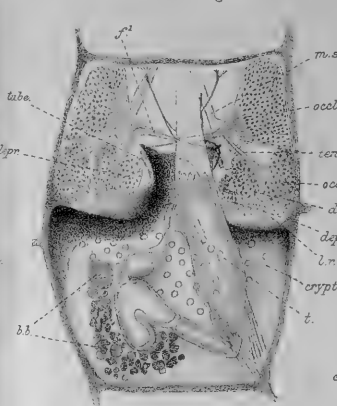


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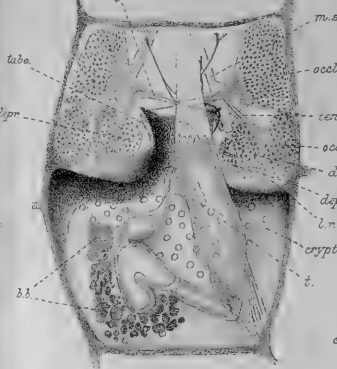


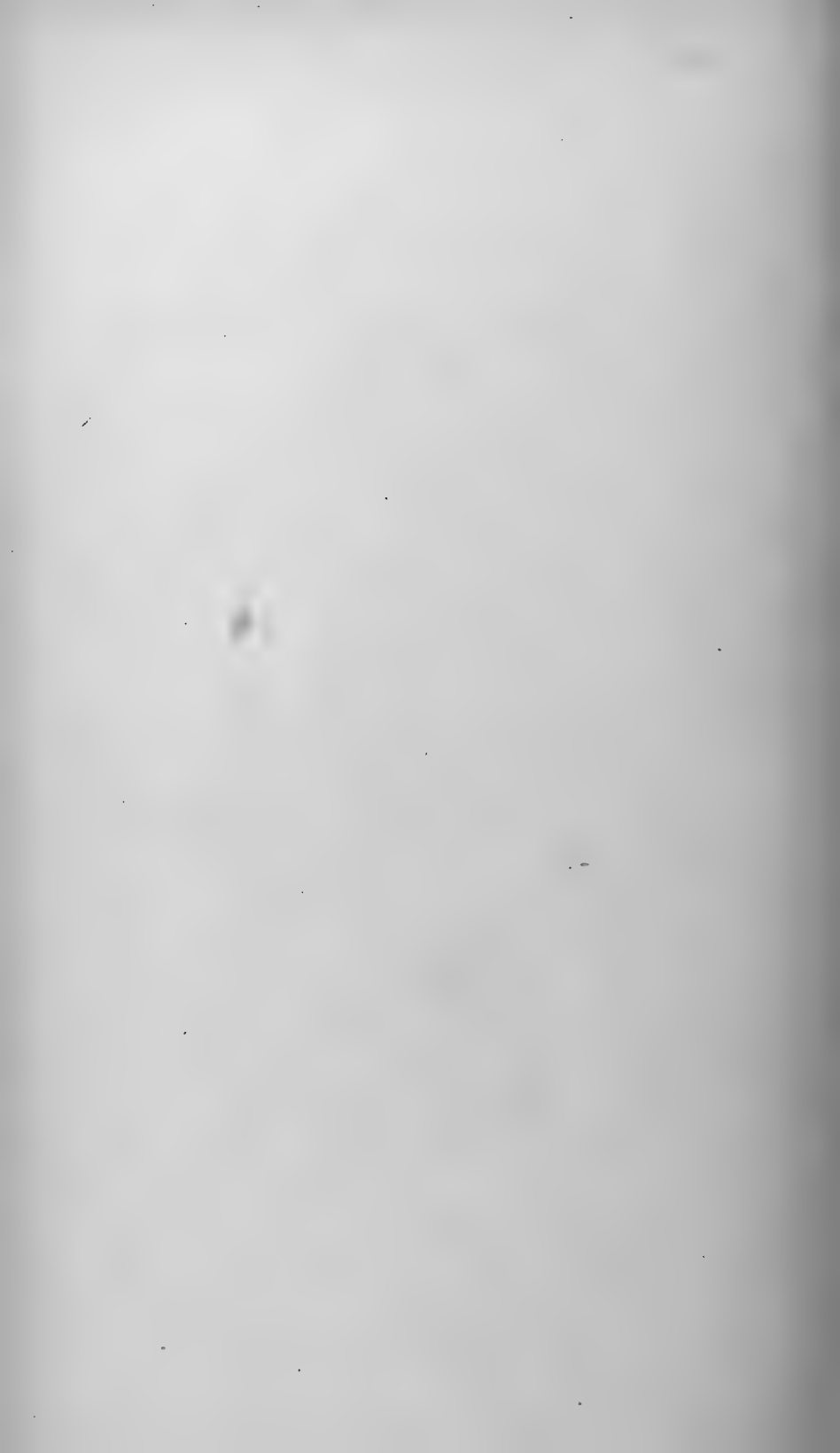
Fig. 65.



Fig. 66.



Huth, Lith. London.



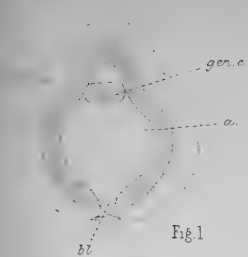


Fig. 1

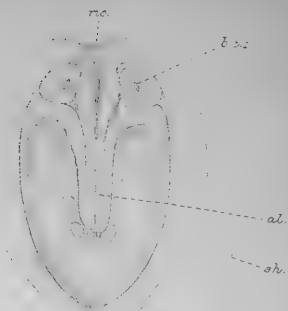


Fig. 3.

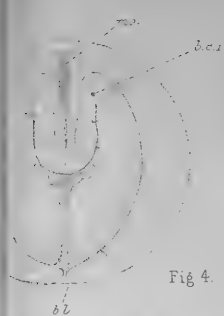


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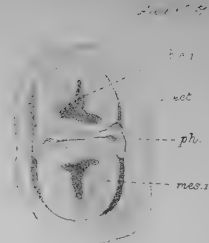


Fig. 5.



Fig. 6.

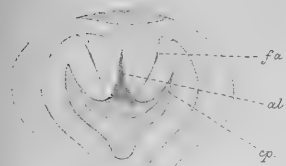


Fig. 2.

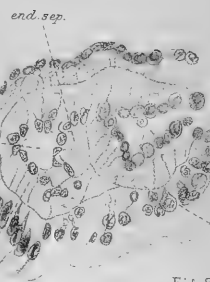


Fig. 8

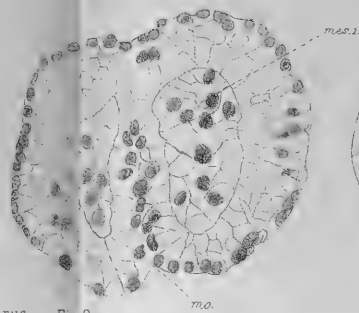


Fig. 9.

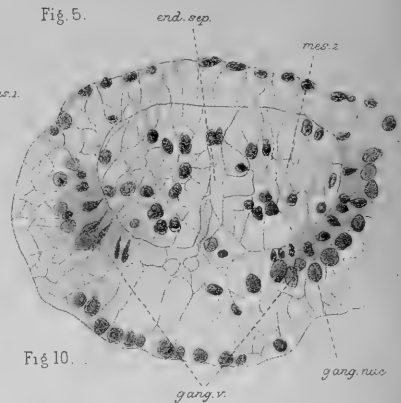


Fig. 10.

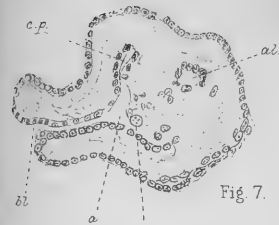


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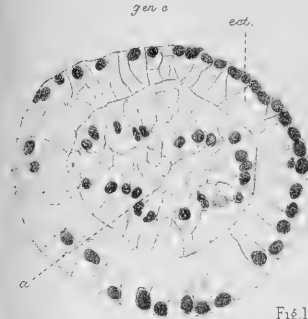


Fig. 12.



Fig. 11.

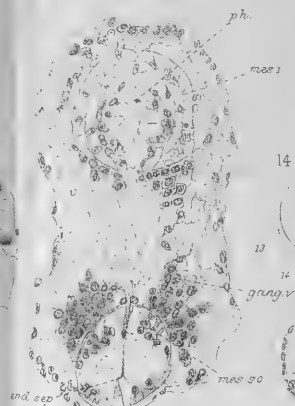


Fig. 13.

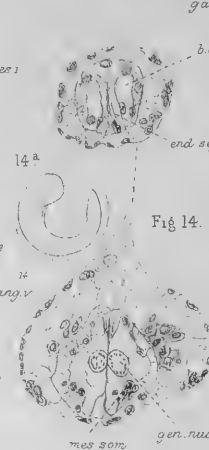


Fig. 14.

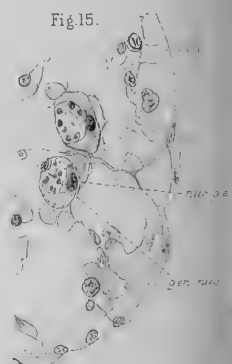


Fig. 15.



Fig. 16.

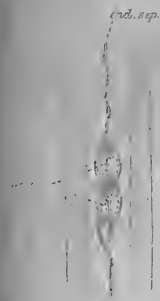
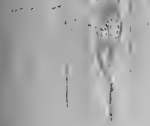


Fig. 17.



D. 14

Fig. 18.

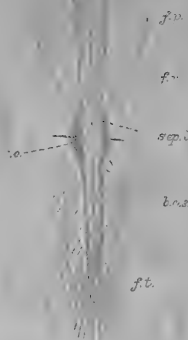


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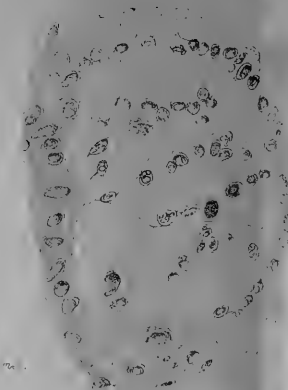


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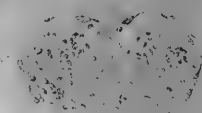


Fig. 27.

Fig. 20.

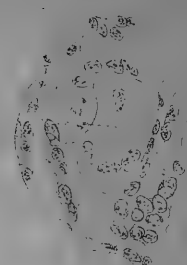


Fig. 23.



Fig. 24.



Fig. 25.



Fig. 26.

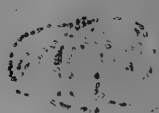


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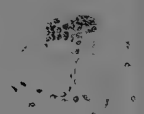
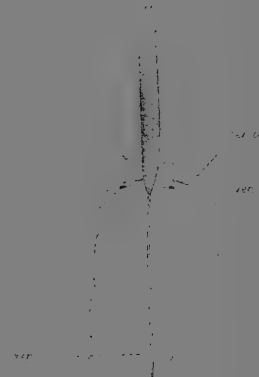


Fig. 29.

Fig. 21.





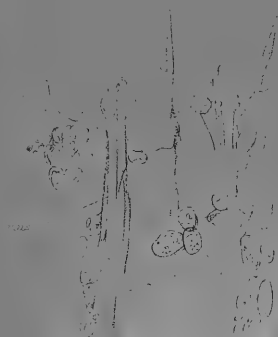


Fig. 30.



Fig. 31

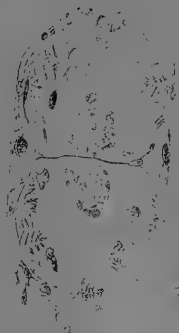


Fig. 32

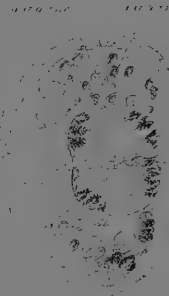


Fig. 33



Fig. 34

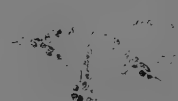


Fig. 35



Fig. 36.



Fig. 38

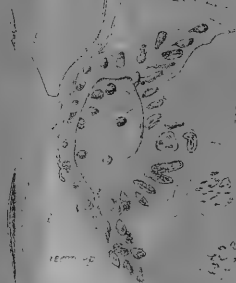


Fig. 39

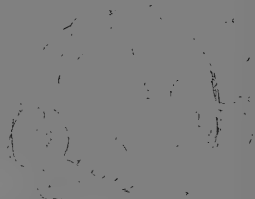


Fig. 41

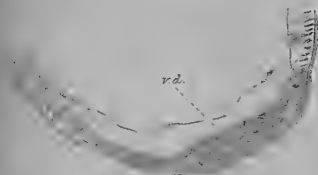


Fig. 37.

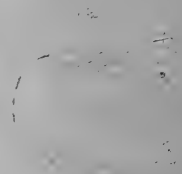
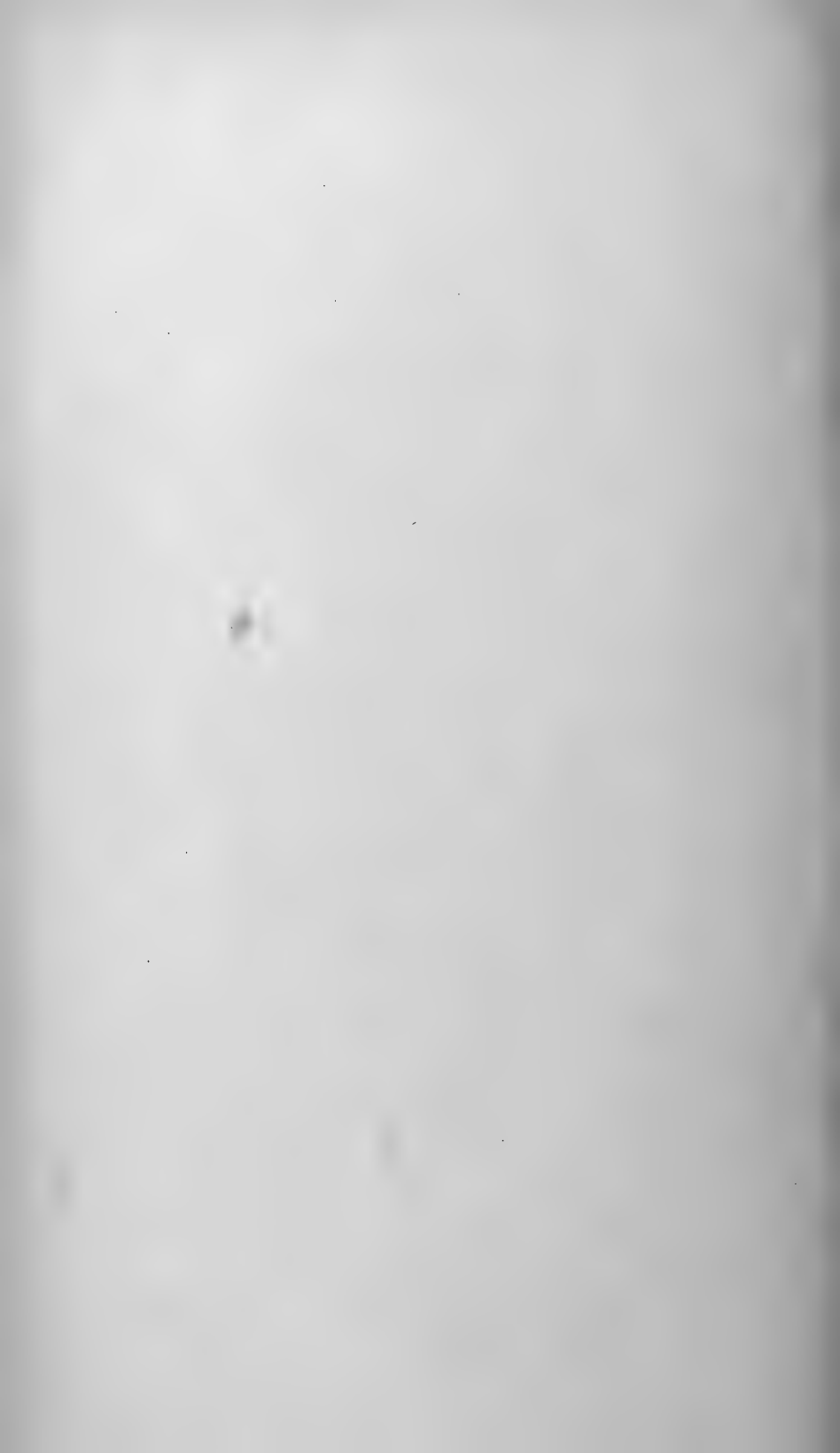


Fig. 42



Fig. 40



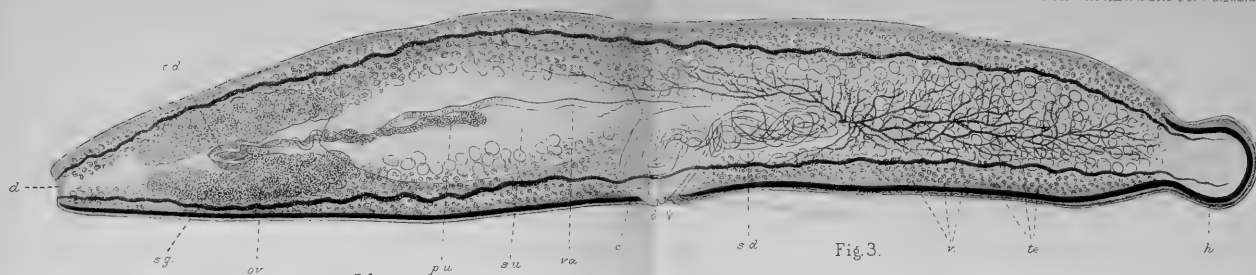


Fig. 3.



Fig. 1.

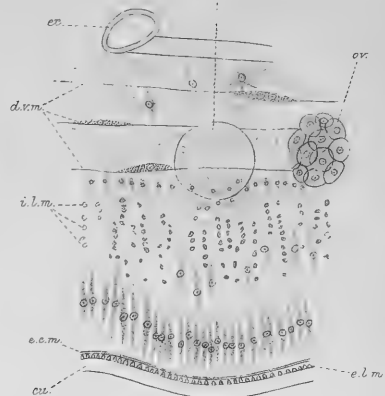


Fig. 4.



Fig. 2.

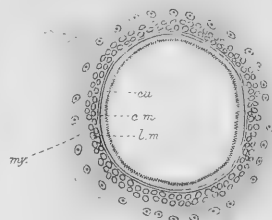


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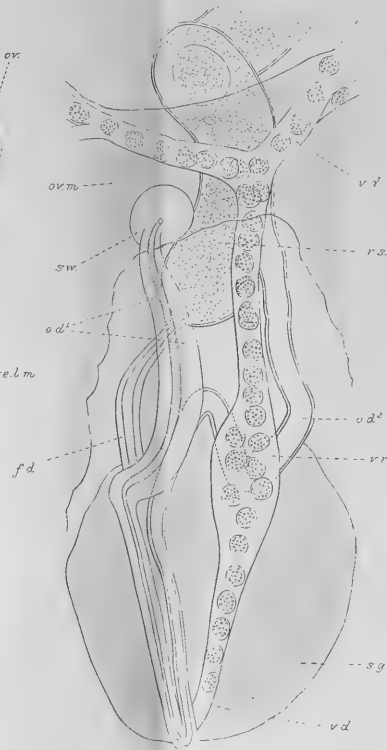


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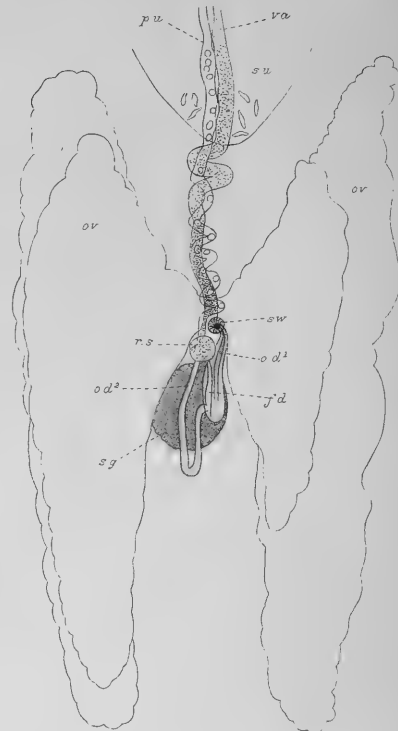


Fig. 6.



Fig. 8



Fig. 9.

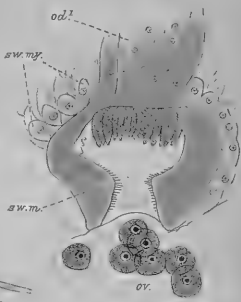


Fig. 10.

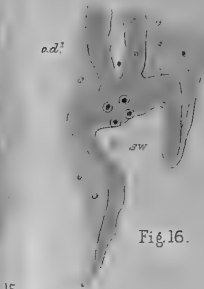


Fig. 12.

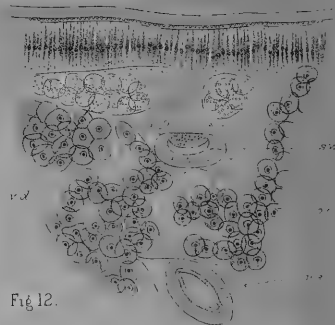


Fig. 13:

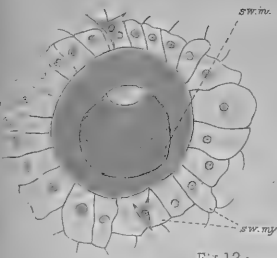


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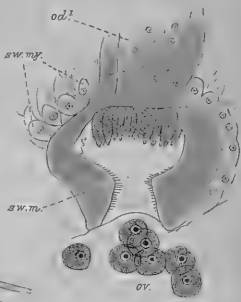


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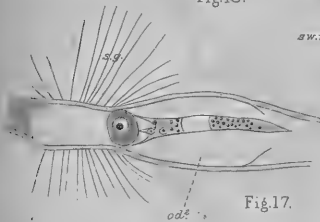


Fig. 16.

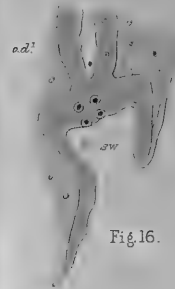
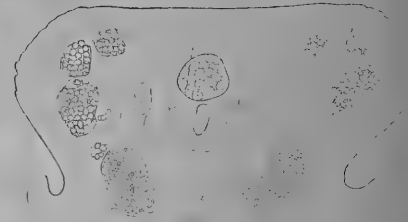


Fig. 19





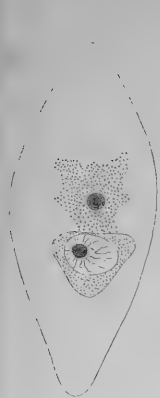


Fig. 19.

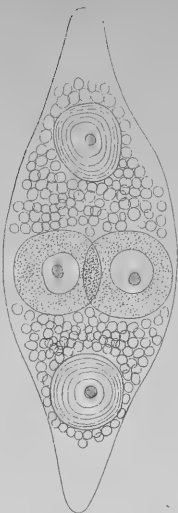


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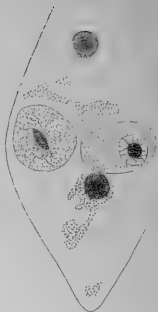


Fig. 21.



Fig. 22.

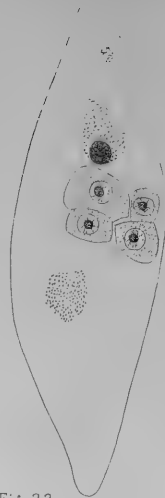


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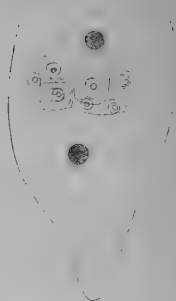


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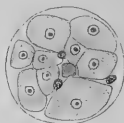


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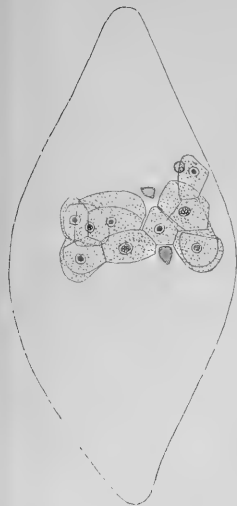


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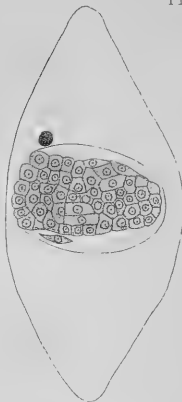


Fig. 27.

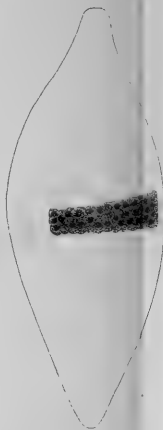


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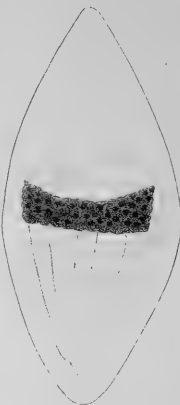


Fig. 29.



Fig. 30.



Fig. 31.

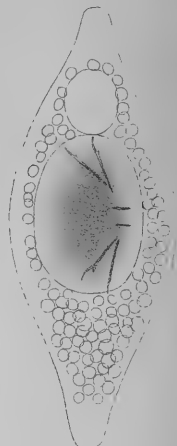
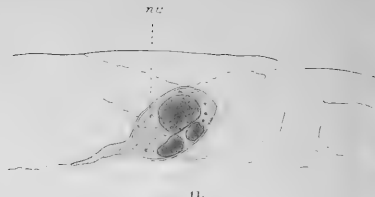


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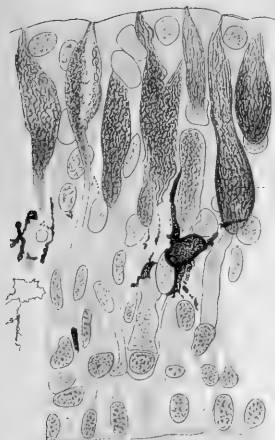




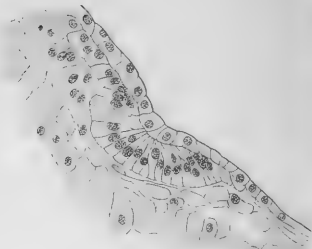
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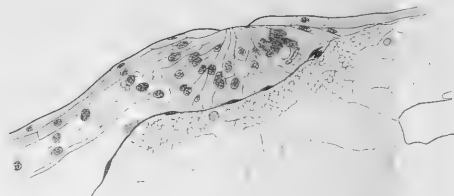
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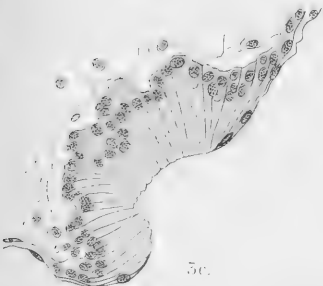
2



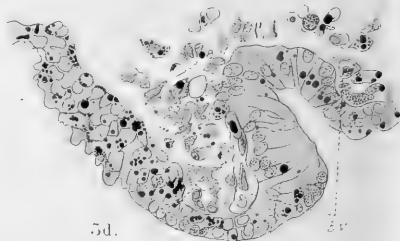
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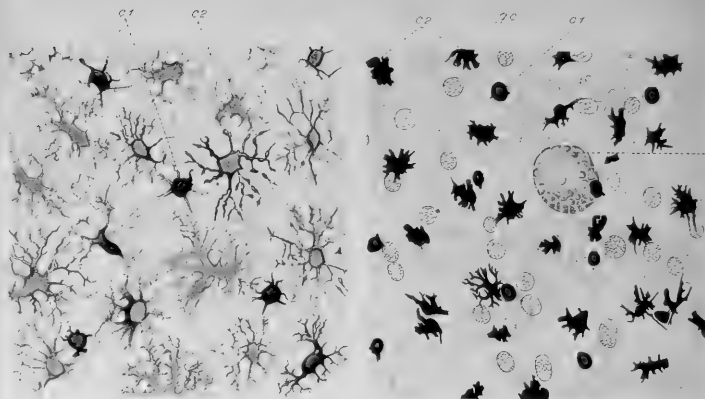
3b.



5c.

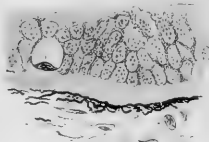


5d.

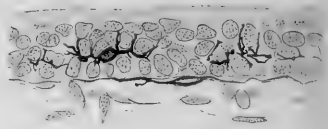


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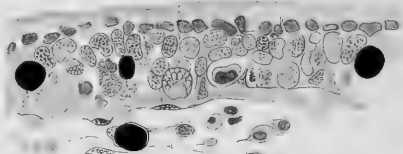
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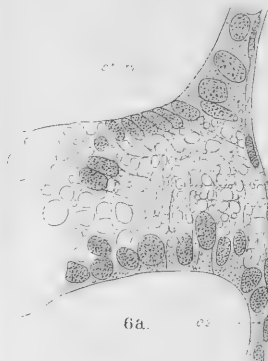
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5b.



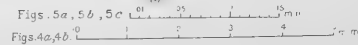
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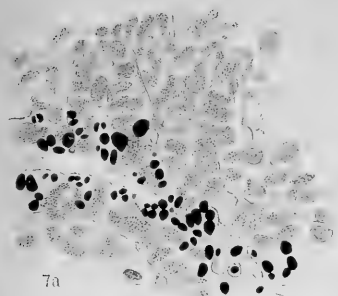
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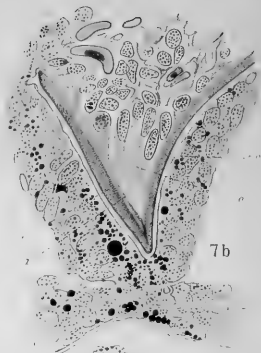
6b.



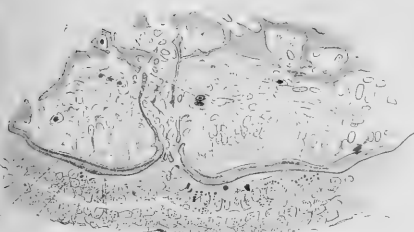




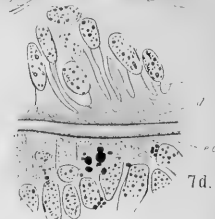
7a



7b



7c



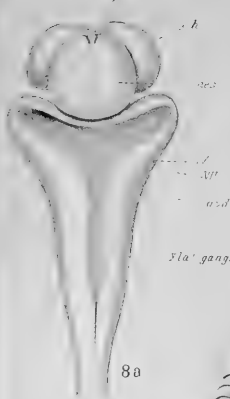
7d



7e



7f



8a



8c



8d



8b



9

Figs 7a, 7b 0.5 1 15 m.m.

Fig 7c 0 1 2 3 m.m.

Fig 7d 0 0.5 1 1.5 m.m.

Figs 7e, 7f 0 1 2 3 4 5 m.m.

Figs 8a, 8b 0 1 2 m.m.

Xvisc gang

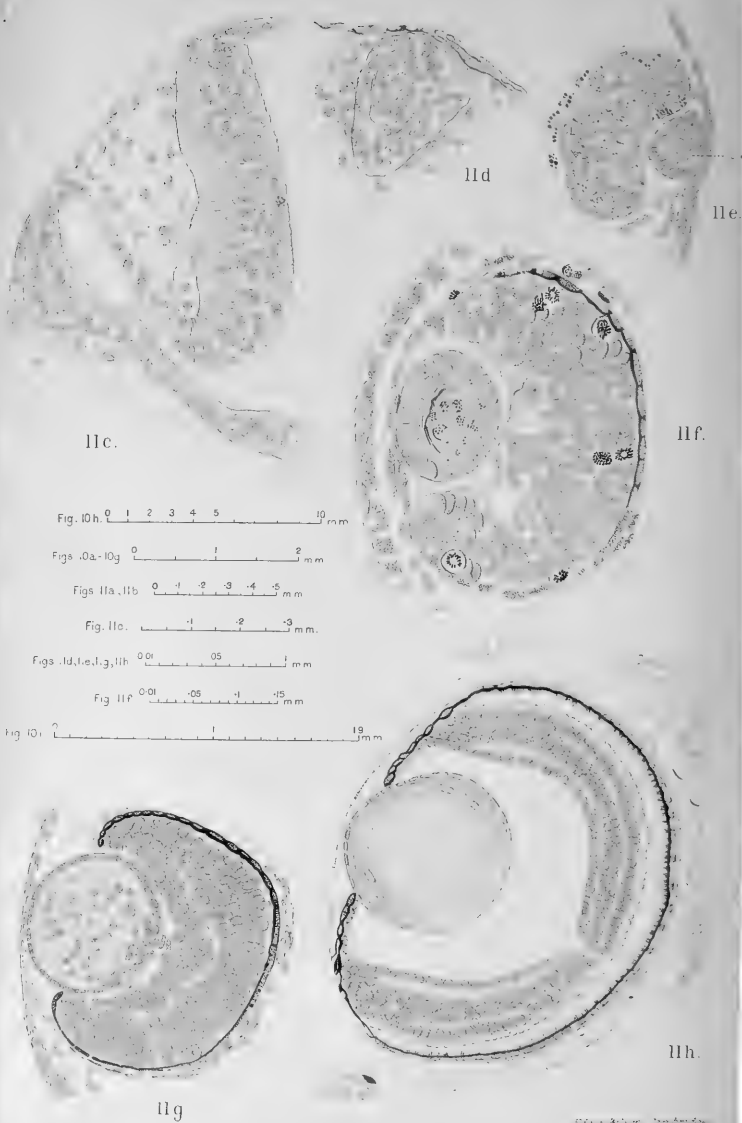
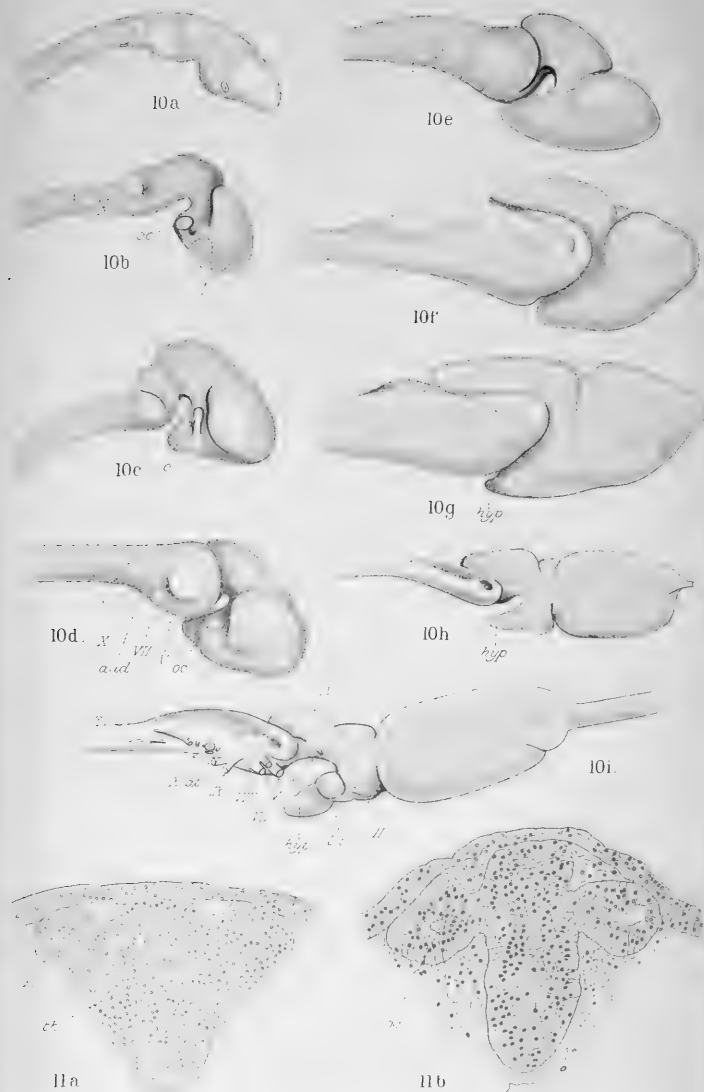
Xvisc gang

Scale

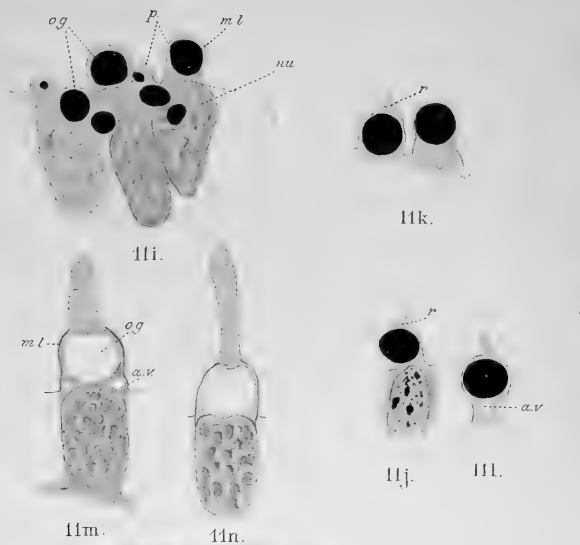
1:1

Drawn by the artist









Figs 11i, 11k, 11m, 11l, 11j 11l

Fig. 12a

Figs. 12b, 12c

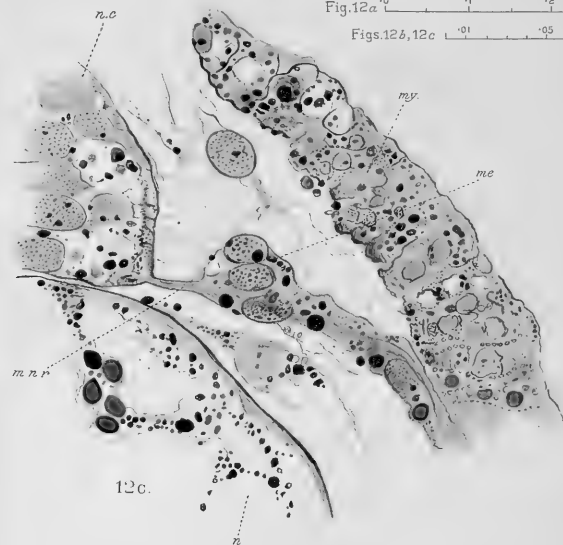
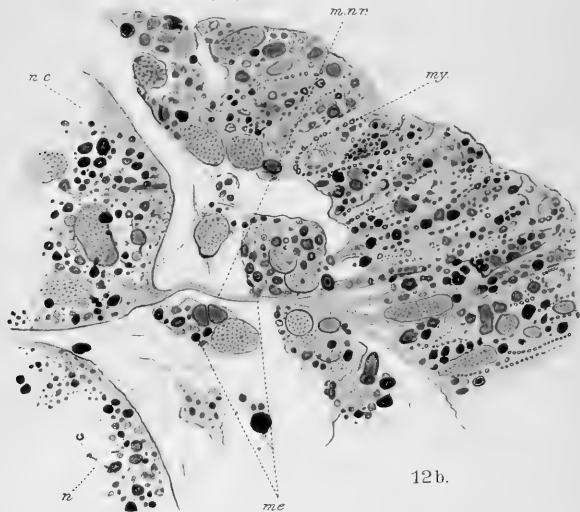






Fig. 1.

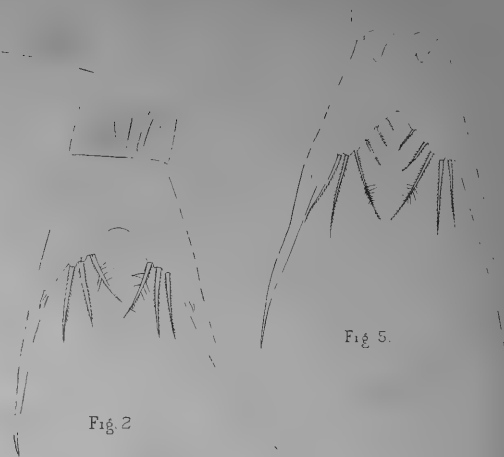


Fig. 2.

Fig. 5.

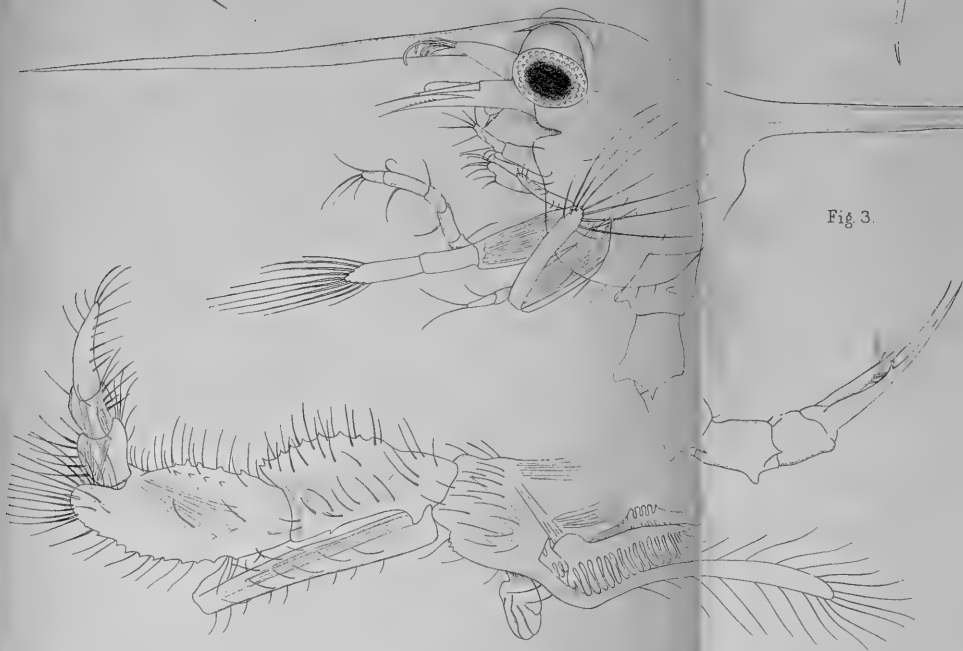


Fig. 3.

Fig. 6.



Fig. 4.

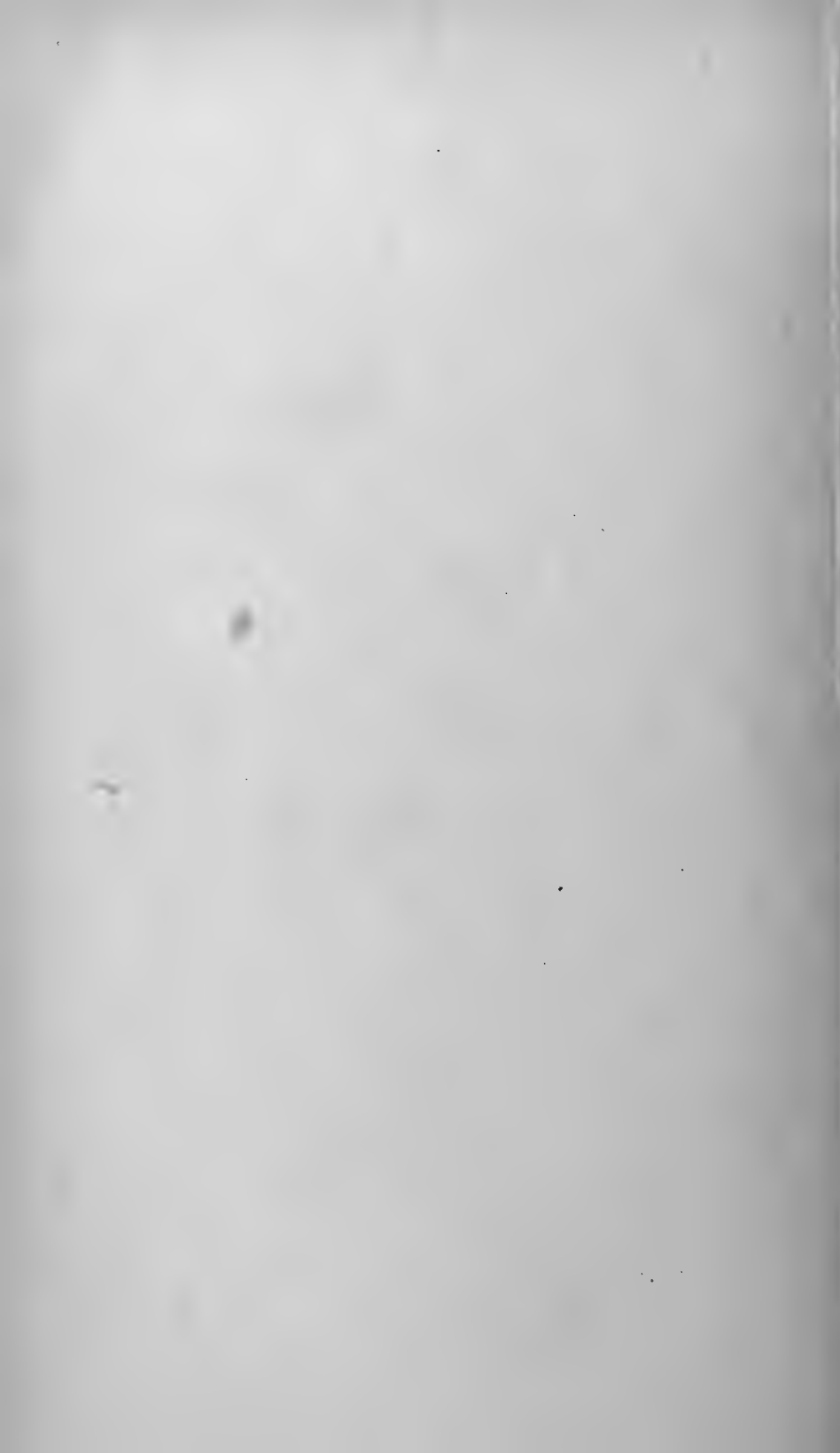




Fig. 8.

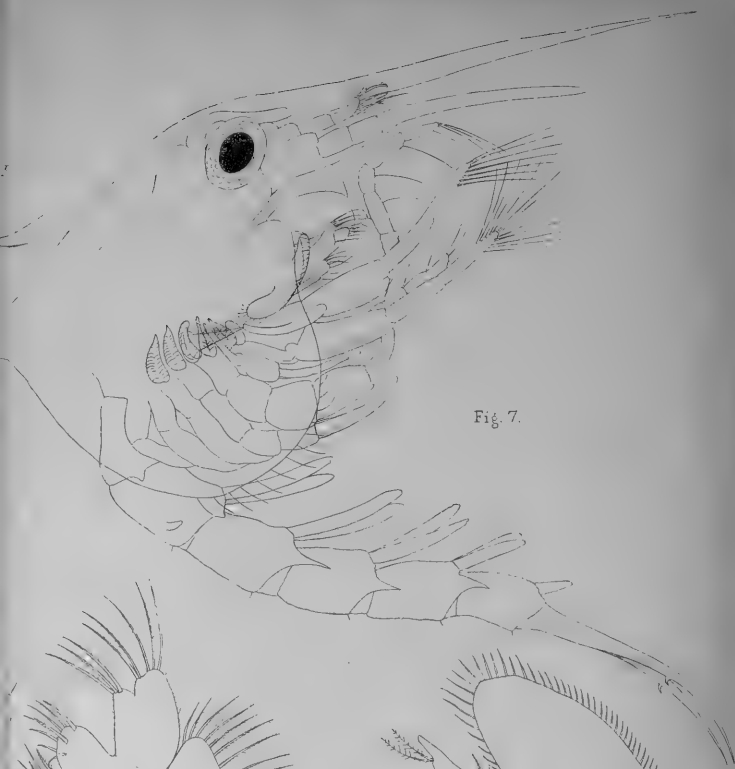


Fig. 7.



Fig. 10.

Fig. 9.

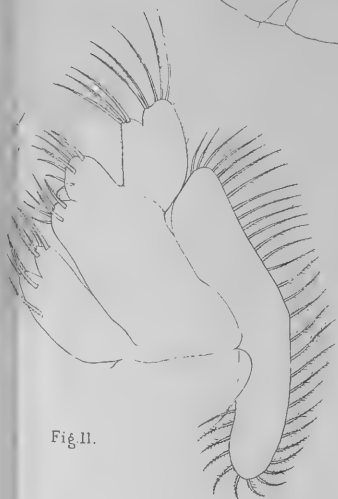


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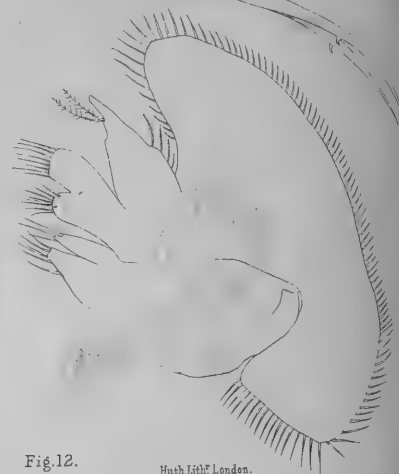


Fig. 12.

Huth, Lith. London.



Fig 17

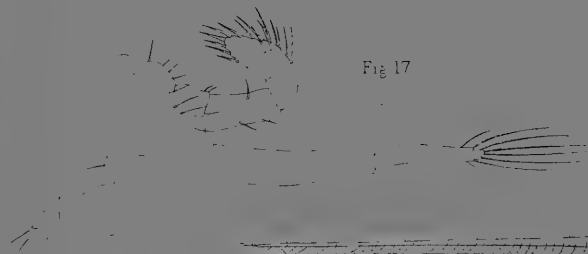


Fig 16

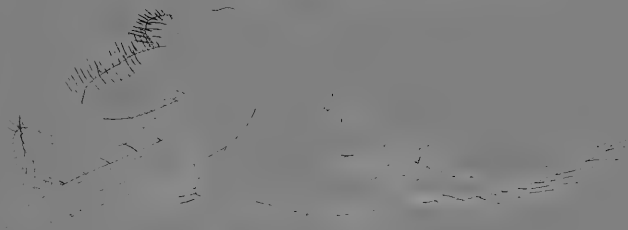


Fig 13

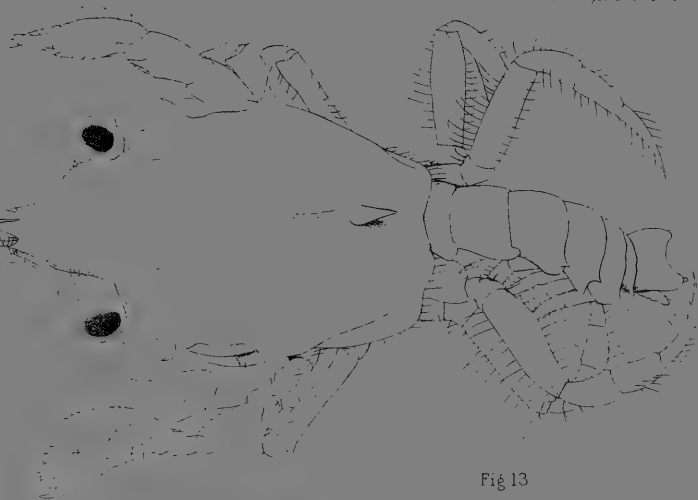


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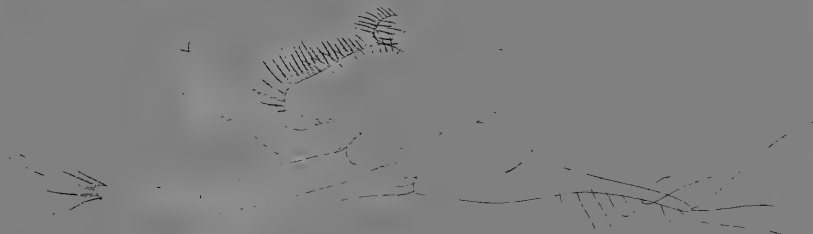
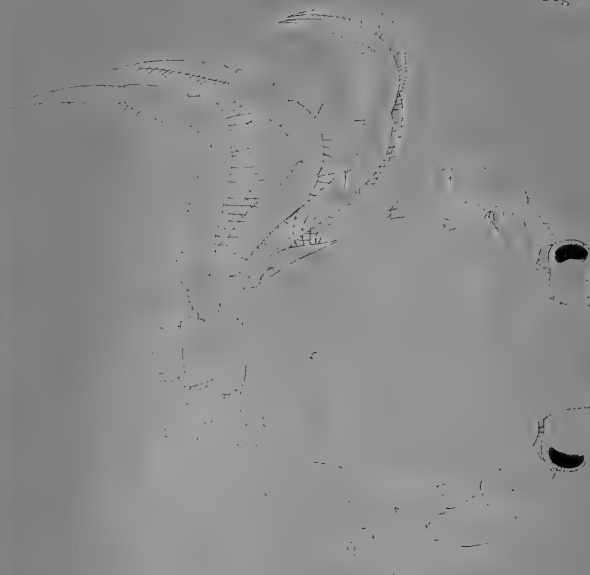
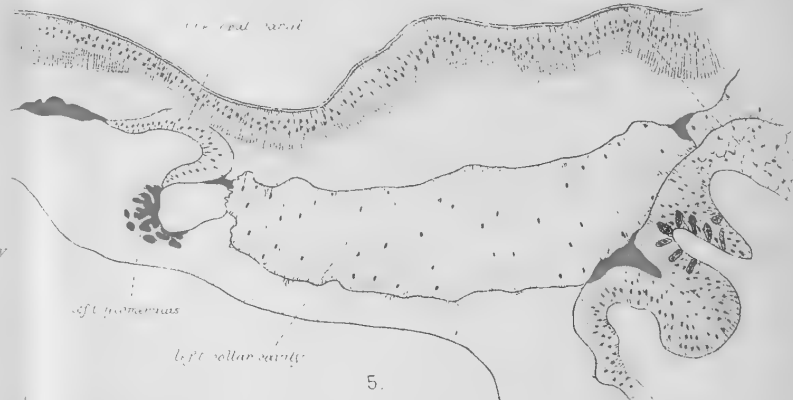
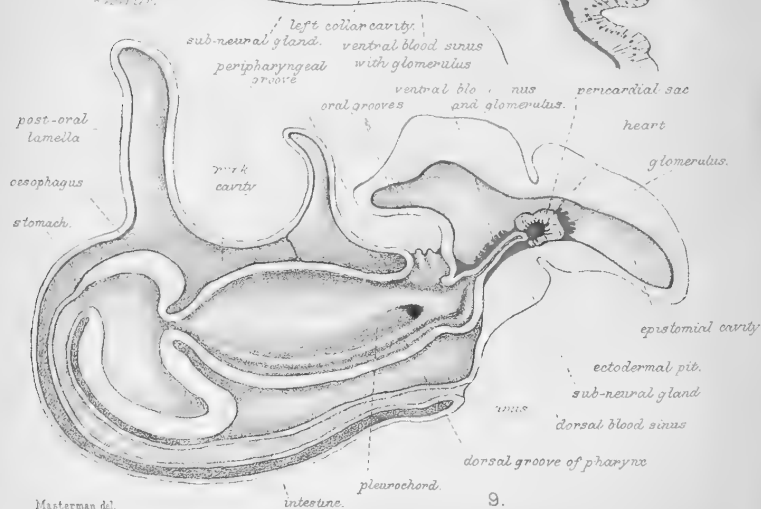
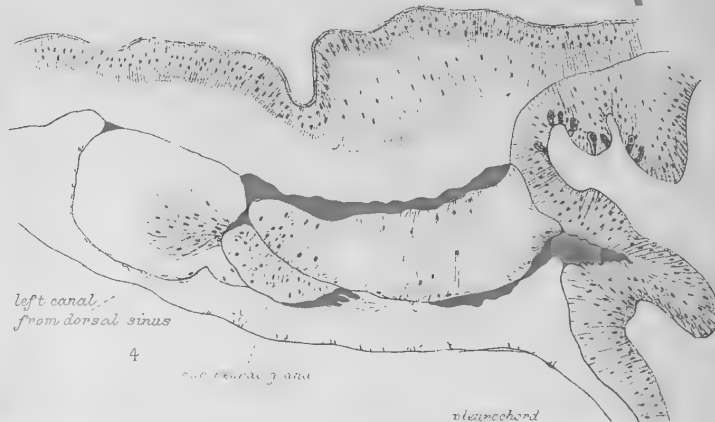
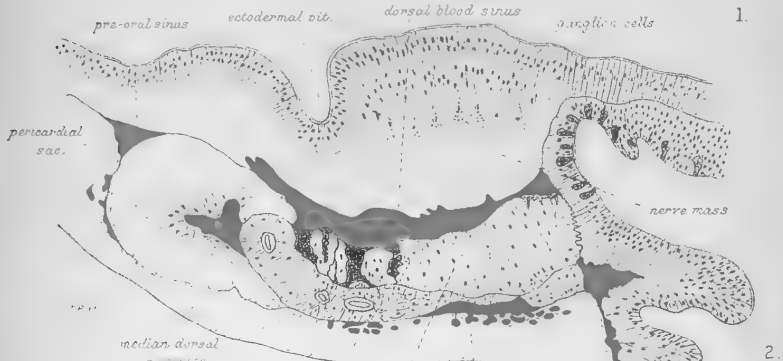
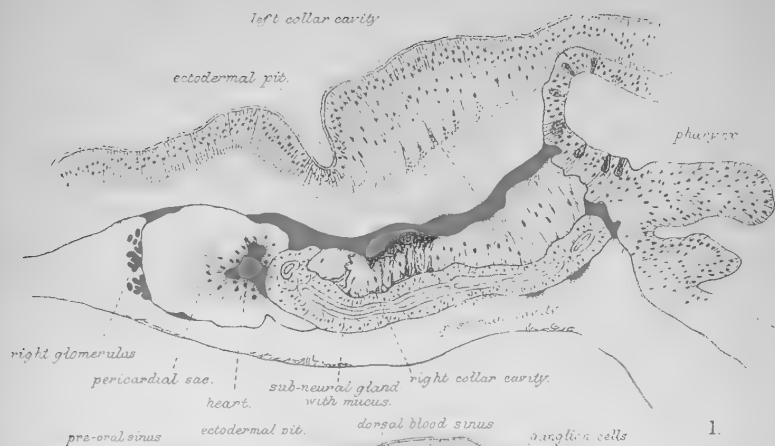
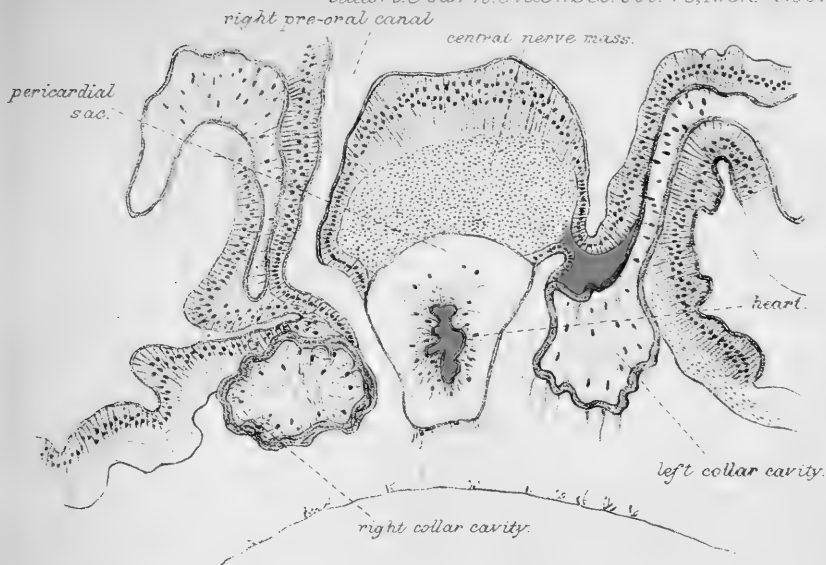


Fig 14

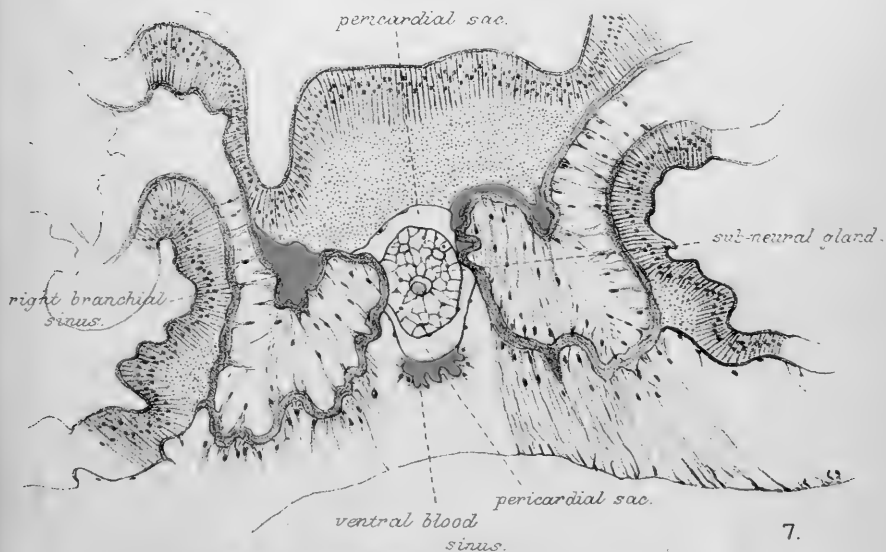








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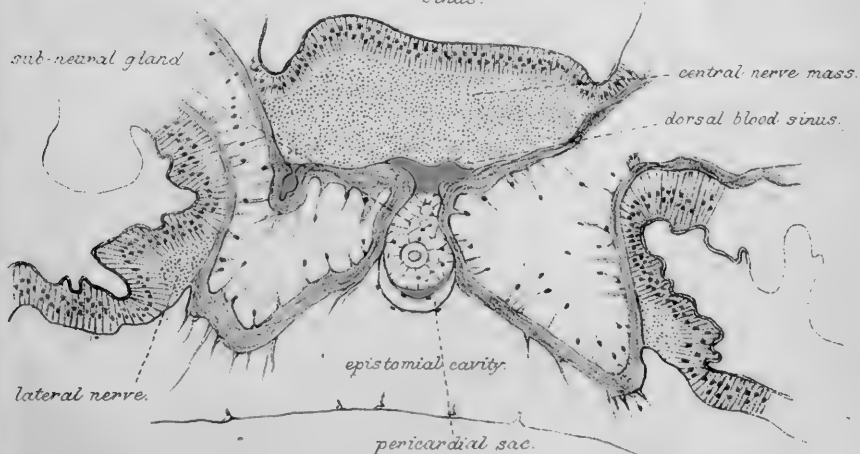






Fig. 1

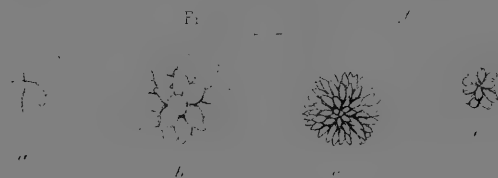


Fig. 2

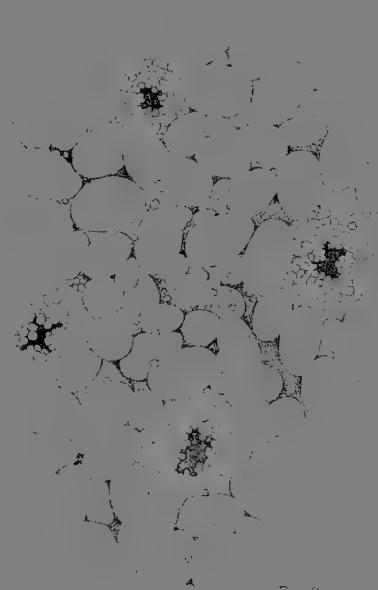


Fig. 3

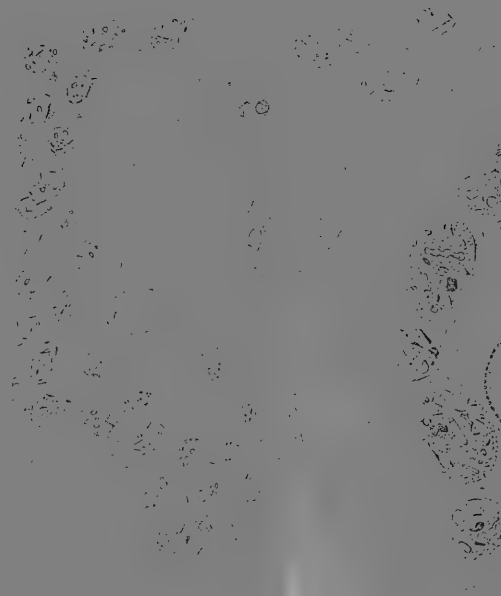


Fig. 4

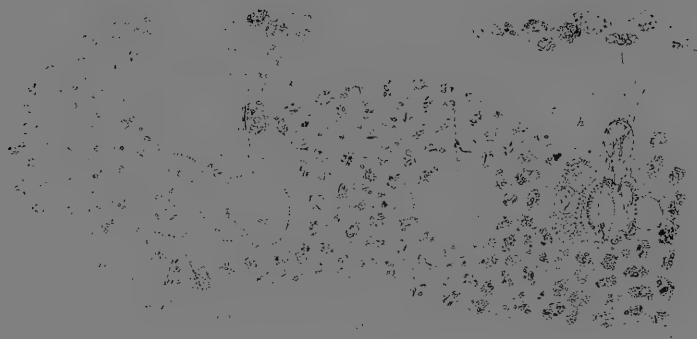


Fig. 5

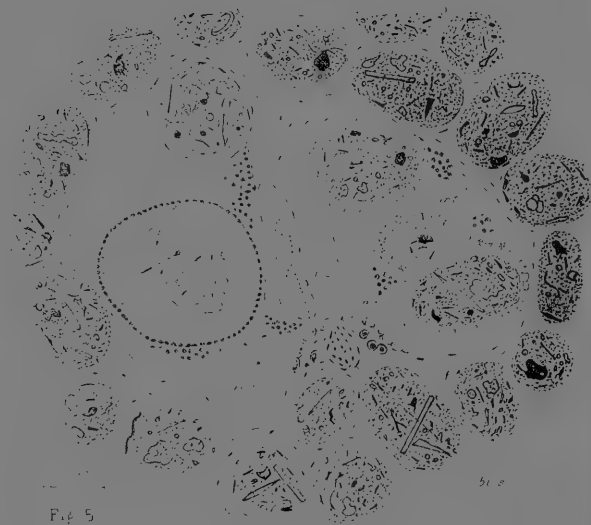


Fig. 6

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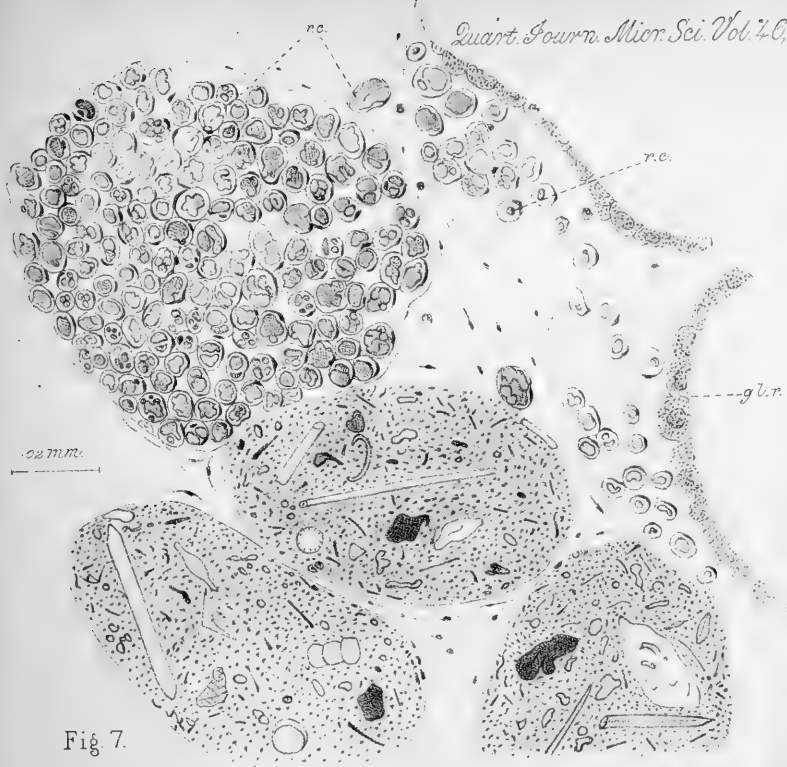


Fig 7.

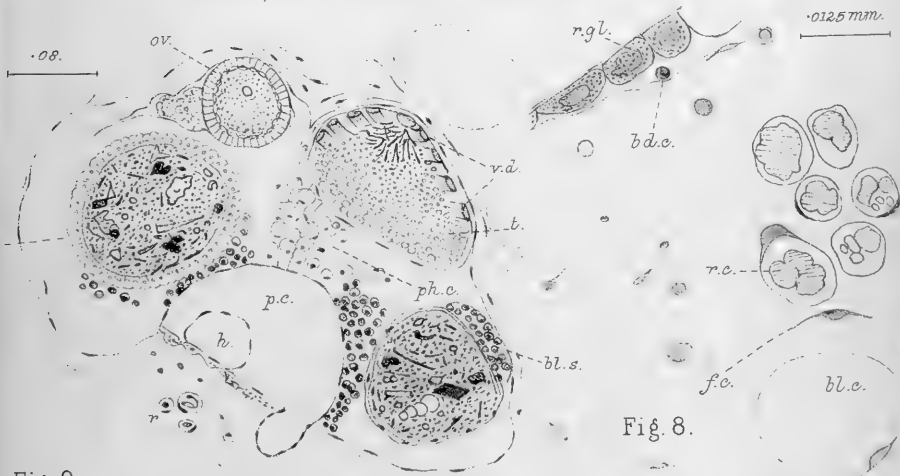


Fig 8.

Fig. 9.

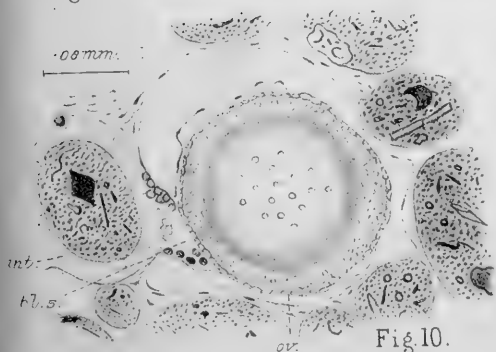


Fig 10.

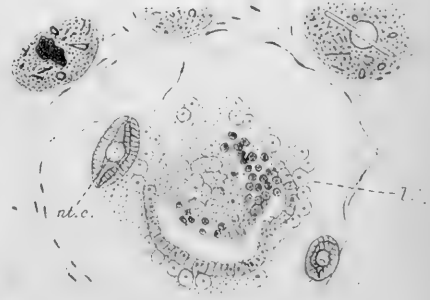
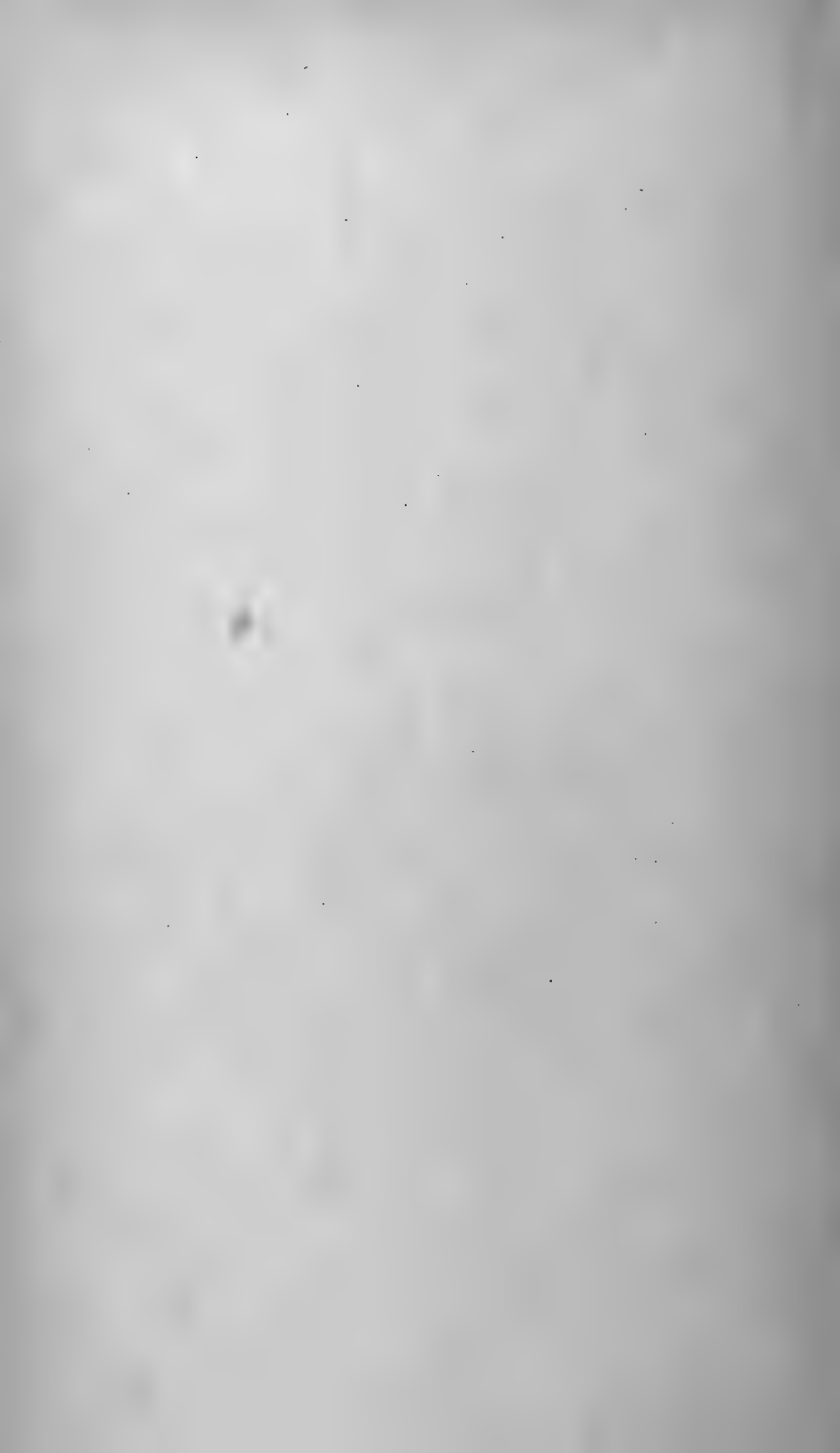
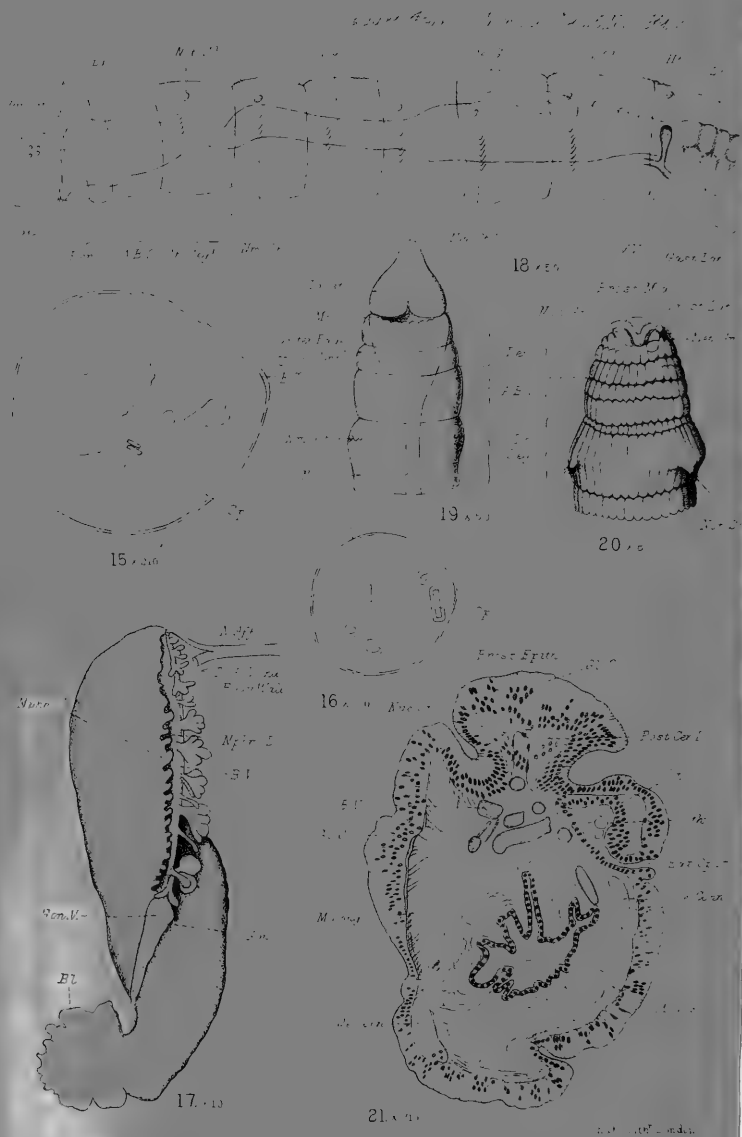
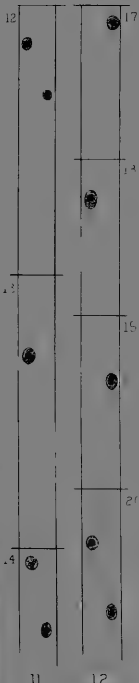
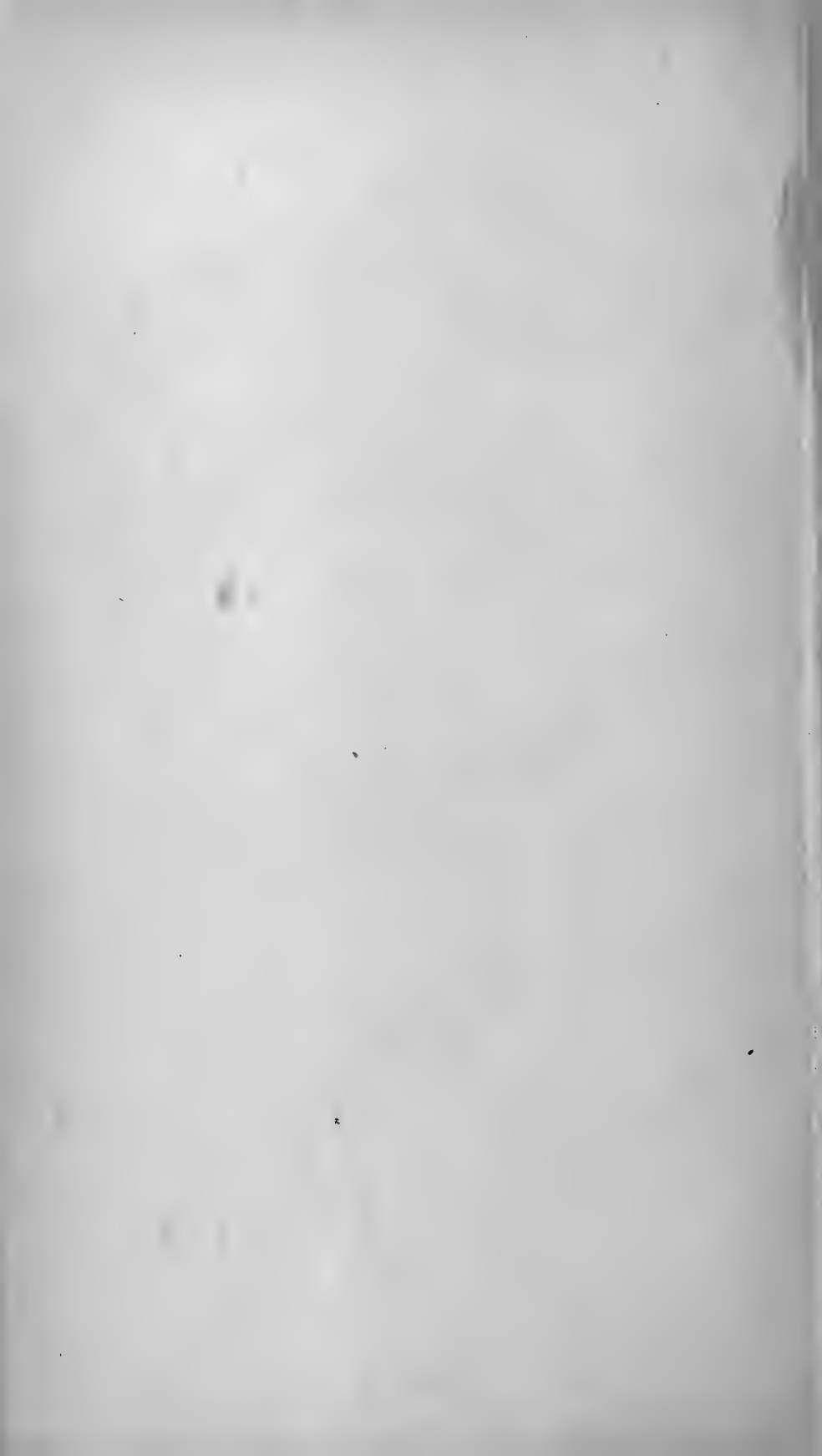
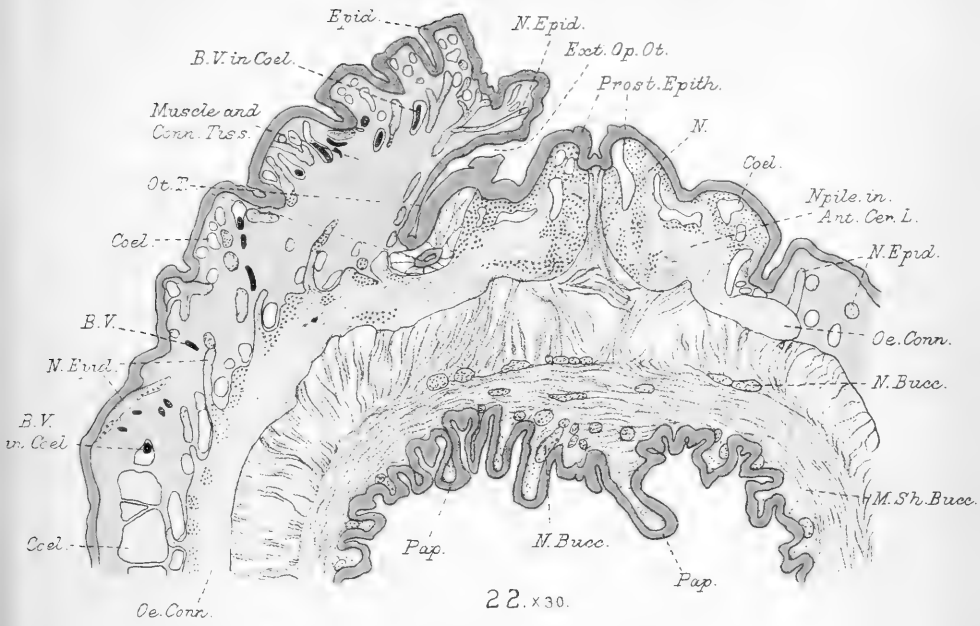


Fig 11.











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